A Genetic Linkage Study of Bipolar Disorder and 13 Markers on Chromosome 11 Including the D_2 Dopamine Receptor

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Chromosome 11 is a region of great interest in the search for genes for bipolar disorder. Although an initial report of linkage to 11p15 was not replicated in numerous subsequent studies, the remainder of the chromosome contains a variety of interesting candidate genes and regions. These include the D_2 dopamine receptor and the site of a chromosomal translocation that has been reported to be associated with bipolar disorder. As part of a systematic survey of the genome for markers linked to bipolar disorder, we have examined 13 markers on chromosome 11 in three large Icelandic families and Amish pedigree 110. No clear evidence of linkage was obtained. The highest lod score was found at D11S29 (lod = 1.63 at $\theta = 0.1$), which is in the general region of the reported translocation breakpoints. However, this lod is not statistically significant, and its meaning is further mitigated by strongly negative lods in two nearby flanking markers. Linkage to the D_2 dopamine receptor locus was strongly excluded (lod = -4.02 at $\theta = 0.0$). In two-point analyses, linkage to bipolar disorder could be excluded to eight of the 13 markers. Multipoint analyses, similarly, failed to reveal any evidence of linkage.
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KEY WORDS: *Bipolar disorder; Linkage; Chromosome 11; D_2 dopamine receptor*

Chromosome 11 has been a region of great interest for linkage studies of bipolar disorder. In 1987, Egeland et al. reported linkage of bipolar disorder to two restriction fragment length polymorphism (RFLP) markers on chromosome 11p15 in a large Old Order Amish pedigree (Egeland et al. 1987). However, numerous subsequent studies in a variety of other populations were unable to replicate this finding (Hodgkinson et al. 1987; Gill et al. 1988; Detera-Wadleigh et al. 1987; Mitchell et al. 1991; Mendlewicz et al. 1991; Nanko et al. 1991; Byerley et al. 1992). Furthermore, subsequent studies in an expanded and updated version of the original Amish pedigree (Kelsoe et al. 1989; Law et al. 1992), as well as a second lateral extension to the pedigree (Pauls et al. 1991) also failed to replicate this finding. Despite this frustrating experience with 11p15 markers, the remainder of chromosome 11 contains a number of interesting candidate genes and candidate regions for bipolar disorder.

The D_2 dopamine receptor gene (DRD2), on the long arm of chromosome 11, is of particular interest because of the antimania effects of neuroleptic medication, the manialike effects of amphetamine, as well as

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reports of changes in cerebrospinal fluid metabolites of dopamine in manic patients (Jimerson 1987). Near DRD2 on 11q is the gene for porphobilinogen deaminase, which has been implicated in both association and linkage studies of schizophrenia and schizoaffective disorder (Sanders et al. 1991; Su et al. 1991; Owen et al. 1991). The 11q region is also interesting because of three reports of families in which bipolar disorder or schizophrenia appears to be linked to a chromosomal translocation with breakpoints in this region (Smith et al. 1989; St. Clair et al. 1990; Holland and Gosden 1990). Although two of the families are affected primarily with schizophrenia rather than bipolar disorder, and the breakpoints appear to be at different sites (11q22.3, 11q21, and 11q25); this is nevertheless an intriguing clue.

In addition to these genes and regions, there are a number of other candidate genes that have been mapped to chromosome 11. These include the D_4 dopamine receptor (Gelernter et al. 1991), tyrosine hydroxylase (Craig et al. 1986), tryptophan hydroxylase (Craig et al. 1991), the M1 muscarinic receptor (Bonner et al. 1991) and the M4 muscarinic receptor (Grewel et al. 1992). We are currently surveying the genome systematically and examining candidate genes in search of loci linked to bipolar disorder in affected families. As part of this survey, we present data from 13 markers on chromosome 11.

METHODS

Subjects

Families from two separate population isolates were used for this study. These include three large families from Iceland and Old Order Amish pedigree 110.

Iceland presents a valuable opportunity for genetic studies of psychiatric illness. Approximately 250,000 people live in Iceland, and of these, 125,000 live in the Reykjavik area. The majority of the population derive from a wave of immigration from Norway approximately 1100 years ago. Because of its isolation and traditions, there has been only a limited immigration since that time. Icelanders are proud of their Viking heritage and actively preserve genealogical knowledge. Large families with multiple generations living in relative proximity are readily available. Similar to Scandinavian countries, Iceland has a national health service with centralized records, which facilitate genetic studies. The epidemiology of affective disorders in this group has been extensively studied by Helgason (1979), and bipolar disorder was found to have a similar lifetime prevalence (0.79%) as that in other populations.

Icelandic families were ascertained from admissions to the psychiatry service at the National University Hospital. These three families were not systematically ascertained, but rather selected through a review of hospital admissions and records for their size, number of affected members, and availability. One of us (HK) has translated the Schedule for Affective Disorders and Schizophrenia (SADS-L) (Endicott and Spitzer, 1978) into Icelandic and confirmed this translation by back translation into English. All subjects were interviewed by one interviewer (HK) using this instrument. In addition to the interview, information was obtained from other family informants for each patient. The interview data, information from other family informants, and available medical records were then used to make research diagnostic criteria (Spitzer et al. 1978) diagnoses. Because of the centralized nature of Icelandic health care and the relative proximity of relatives to each other, medical records and family informants were available for almost all patients. Diagnoses were made on deceased or unavailable members using medical records and family informants, but in all the linkage analyses described here, they were considered to have an unknown diagnosis. The resulting pedigrees are presented here for the first time in Figure 1. Blood samples were obtained for preparation of deoxyribonucleic acid (DNA) and lymphoblastoid cell lines.

Affective disorders in the Old Order Amish have been studied in detail by Egeland et al. (1987), and the advantages of this population for genetic research previously reviewed (Egeland and Hostetter 1983). Amish pedigree 110, as used in this study, includes 118 individuals, 22 of whom have bipolar disorder and 7 who have recurrent unipolar depression. Pedigree 110 was selected through a systematic survey of families segregating with major affective disorder ascertained from the Old Order Amish living in Lancaster County, Pennsylvania. Diagnoses were obtained by review of the SADS-L interview of the subject, interviews of multiple informants regarding the subject, and abstracted medical records by a panel of five psychiatric clinicians blind to the subject's identity or genotypes (Hostetter et al., 1983). The structure and diagnostic data regarding this pedigree has been published previously (Kelsoe et al. 1989). The pedigree used here is identical to that previously described and diagnoses unchanged, with the exception of two previously unaffected members (GM5989 and GM6003), who have had subsequent onset of bipolar I disorder (NIGMS, 1992).

Genotyping

Information and references regarding the probes and alleles for the 13 loci examined are summarized in Table 1. Three probes were kindly provided by Dr. Ray White; they are p3C7, pHBI18P2, and pMCT128.1. All other probes were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The probe for the D_2 dopamine receptor, pD2-1.7 (DRD2), was a 1.7-kb fragment that was subcloned from λHD2G1

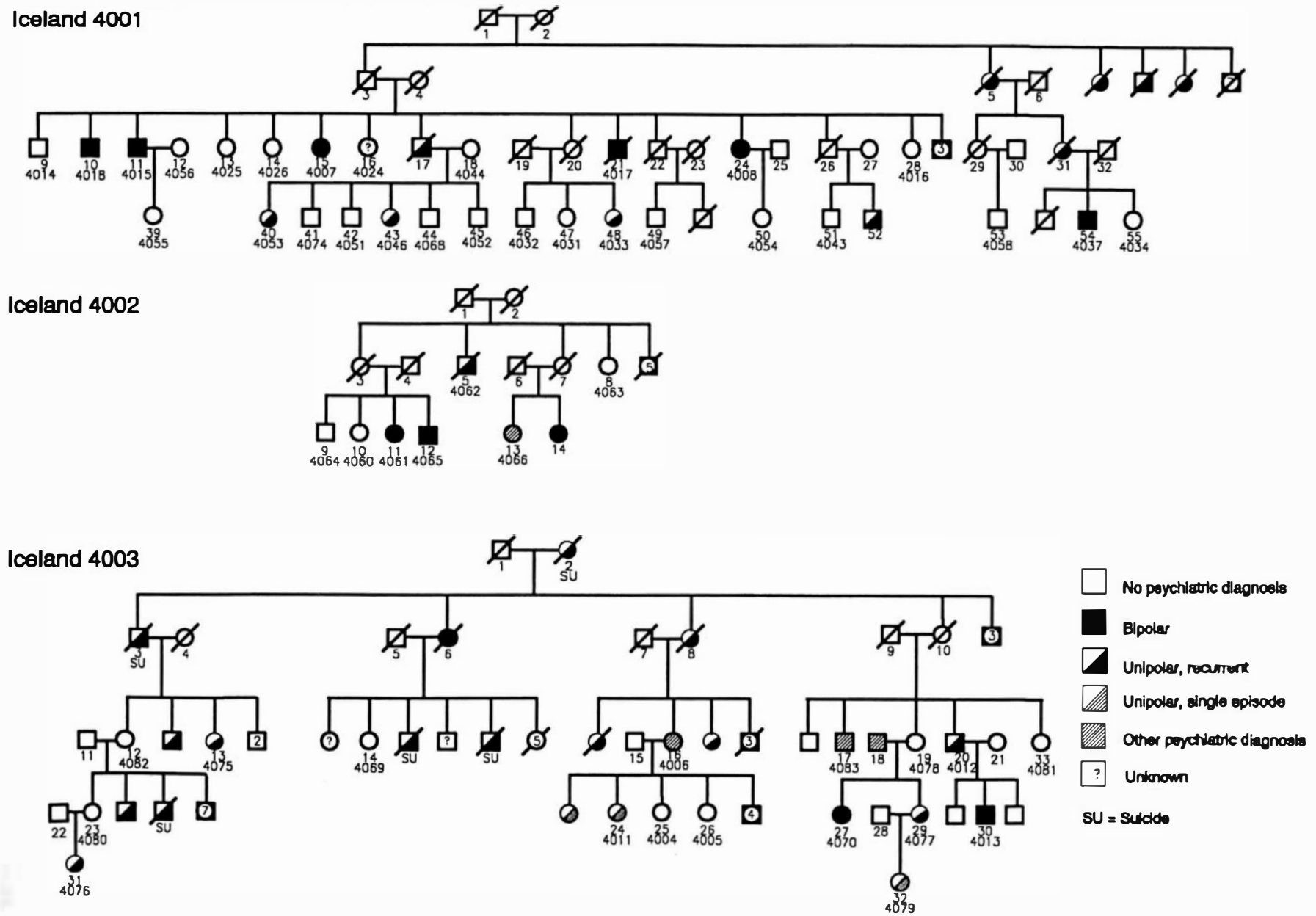

Figure 1. Three Icelandic pedigrees used for linkage studies of bipolar disorder. Symbols for various diagnoses are indicated. Deoxyribonucleic acid was available for individuals with a 4000 series ID number under their symbol. Some symbols representing individuals or branches of families that were not available for study have been omitted or collapsed to protect the anonymity of the families.

Table 1. Chromosome 11 Loci Examined

Locus	Probe	Enzyme	Allele Sizes (kb)	Allele Frequencies	References
HRAS	pEJ6.6	SstI	5.2; 5.7; 6.2; 6.8	0.3; 0.3; 0.1; 0.3	Capon et al. (1983)
PTH	p20.36	TaqI	2.4; 2.5	0.63; 0.37	Anatonarakis et al. (1983)
D11S16	p32-1	MspI	11; (7.6, 6.4, 4); (6, 4, 3)	0.5; 0.18; 0.32	Feder et al. (1985)
CAT	pINT800	TaqI	3.5; (2.5, 1)	0.5; 0.5	Quan et al. (1985)
D11S288	p3C7	MspI	5.7; 3.1	0.72; 0.28	Julier et al. (1990)
D11S97	MS51	PCR	7 alleles 1.4–4 kb	*	Jeffreys et al. (1988)
D11S84	p2-7-1D6	TaqI	4.2; 6.4	0.77; 0.23	Maslen et al. (1988)
DRD2	pD2-1.7	TaqI	3.7; 6.6	0.76; 0.24	Grandy et al. (1989)
D11S144	MCT128.1	MspI	2.6; 2.9	0.48; 0.52	Julier et al. (1990)
D11S29	pL7	TaqI	10.9; 13.9	0.77; 0.23	Warnich et al. (1986)
D11S147	HBI18P2	PstI	4; 5	0.75; 0.25	Julier et al. (1990)
D11S83	ϕ2-25	MspI	4.35; 3.2	0.32; 0.68	Maslen et al. (1988)
D11S419	Mfd58	PCR	112; 114; 116; 118 bp	0.02; 0.52; 0.44; 0.02	J. Weber, personal communication

* Seven different alleles were distinguished for MS51 for both Icelandic and Amish populations, although the alleles differed between the populations. Allele frequencies were estimated separately for each population as described in Linkage Analysis.

(provided by O. Civelli; Grandy et al. 1989) into pBluescript to reduce background from repetitive sequences (Turner et al. 1991).

Restriction fragment length polymorphisms were detected by Southern blotting and hybridization as described previously (Kelsoe et al. 1989). Briefly, DNA was prepared by phenol/chloroform extraction from lymphocytes or lymphoblastoid cell lines (Sambrook et al. 1989). Five to 10 µg of genomic DNA was digested overnight with the appropriate restriction enzyme according to the manufacturer's instructions. The resulting fragments were separated by agarose gel electrophoresis (0.8%) and transferred to nylon membranes by vacuum. Probes were radiolabeled by random primer reaction (Feinberg and Vogelstein 1983), and hybridizations were performed in Hybrisol (Oncor) at 45°C. Membranes were washed three times in 0.1 × standard saline citrate, 0.1% sodium dodecyl sulfate for 15 minutes each at room temperature, and once for 1 hour at 52°C. For probes containing repetitive sequences, human total genomic DNA was included in the hybridization mix or preassociated with the probe (Litt and White 1985). The blots were then placed against Kodak XAR film using enhancing screens (Dupont Lightning Plus) at −80°C for 1 to 3 days.

Two polymorphisms were detected by polymerase chain reaction (PCR)-based methods: MS51 (D11S97) and Mfd58 (D11S419). The MS51 is a highly informative VNTR polymorphism that was detected by PCR amplification of the polymorphic region followed by Southern blotting and hybridization. Hybridization and extended PCR elongation times were required to detect the alleles of higher molecular weight (1 to 4 kb). This procedure was performed essentially as described by Jeffreys et al. (1988) using the same primer sequences that they employed. Briefly, 20 ng of template genomic DNA was amplified in a 10-µl reaction containing 250 nM of each primer, 1.5 unit of Taq polymerase, 200 µM of each nucleotide, and PCR buffer (final concentration: 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM $MgCl_2$, and 0.01 mg/ml gelatin). The thermal cycling was performed as follows: 2 minutes at 95°C, 1 minute at 60°C, and 15 minutes at 70°C. Fifteen cycles of this protocol were performed, followed by an additional annealing period for 1 minute at 60°C and an extension period for 15 minutes at 70°C. The PCR products were then digested with 3 units S1 nuclease at 37°C for 30 minutes in the buffer recommended by the manufacturer (BRL). The resulting fragments were then blotted and hybridized as described above. A PCR-amplified fragment of MS51 was radiolabeled and used as a probe. Seven different alleles were distinguished in the Icelandic families and seven were identified in the Amish family, although the alleles differed between the two populations. Each population was scored and analyzed separately as described below.

The polymorphism Mfd58 is a CA repeat marker that was detected using the primers and method of Weber et al. (J. Weber, personal communication; Weber and May, 1989). One hundred nanograms of genomic DNA was amplified in a 10-µl reaction containing 200 nM of each Mfd58 primer, 200 µM of each nucleotide, 1 unit of Taq polymerase, 0.1 µl [α-^{32}P]-deoxycytidine triphosphate (3000 Ci/mmol; Amersham International) in PCR buffer as described above. The thermal cycling protocol included the following steps: an initial denaturation for 1.5 minutes at 94°C, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 1.5 minutes at 72°C, and a final elongation of 5 minutes at 72°C. The PCR products were denatured by adding 20 µl of stop buffer (95% formamide, 10 mM ethylenediaminetetraacetic acid, 0.1% bromophenol blue, and 0.1% xylene cyanol) and heating for 10 minutes at 96°C. The fragments were then separated by electrophoresis on a 5% polyacrylamide-denaturing sequencing gel at 60W for 3 hours, dried, and placed against Kodak XAR

film with enhancing screens (Dupont Lightning Plus) at −80°C for 1 to 3 days. For both RFLP's and PCR markers, genotypes were scored independently by two raters blind to diagnosis, but open to pedigree structure.

Linkage Analysis

Linkage analyses were performed using the LINKAGE analysis package, V 5.1 (Lathrop et al. 1984). Analyses for all families were conducted under a model of transmission described previously for the Amish family (Kelsoe et al. 1989) and supported by a segregation analysis in that population (Egeland et al. 1987). Specifically, autosomal dominant transmission was modeled with age-dependent reduced penetrance. Penetrance of the disease allele was modeled by five liability classes as shown in Table 2 and ranged from 0.0001 at age 14 to a maximum of 0.85 at age 30. Penetrance of the nonsusceptible genotype increased from 0.0 to a maximum of 0.001 at age 30. The frequency of the disease allele was 0.021.

It is not clear that this is the appropriate model for the Icelandic families. Ideally, a separate segregation analysis would be conducted in this population to determine the best model. Unfortunately, because these families were not systematically ascertained and because of the small sample size, we felt that such an analysis would not be meaningful. Other studies of bipolar disorder have supported an autosomal dominant mode of transmission (Rice et al. 1987). Therefore, we felt that it was reasonable to apply a similar model to the analysis of the Icelandic families.

Marker allele frequencies were drawn from published data for all markers except D11S97 (MS51). Published allele frequencies could not be identified for this locus; furthermore, there were marked differences between allele frequencies in the Icelandic and Amish families. Although a detailed comparison of allele sizes between the two populations was not conducted, it was apparent that there were several alleles present in each of the two populations that were not present in the other. For this reason, allele frequencies for this locus were estimated from unrelated marry-in spouses, separately for each population, and analyses were conducted separately.

Individuals with diagnoses of bipolar disorder (bipolar I and bipolar II), schizoaffective disorder or recurrent major depression were considered affected. Those with a single episode of major depression or any other psychiatric diagnosis were considered unknown. All individuals who were not interviewed directly were considered to have unknown diagnoses.

Two-point analyses were conducted using the MLINK program. The one inbreeding loop present in Amish pedigree 110 was broken by doubling individual GM5995. All possible marker-to-marker two-point analyses were performed, and the results were consistent with published maps (data not shown). Multipoint analyses were performed using the LINKMAP program to move the affection status locus across a fixed map of the markers based on published map data (Junien and van Heyningen 1990; Julier et al. 1990; and CEPH Database, V 5.0). Analyses of more than two fixed loci in addition to the disease locus were not feasible because of the large amount of computer time required. Therefore, these analyses were conducted an interval at a time as three-point analyses. Published map data were not available for one marker Mfd58 (D11S419), which was therefore not included in the multipoint analyses.

Table 2. Liability Classes for Age-Dependent Penetrances of Bipolar Disorder

Age (yrs)	AA	Aa	aa
0–14	0.0001	0.0001	0.0
15–19	0.18	0.18	0.0001
20–24	0.42	0.42	0.001
25–29	0.73	0.73	0.001
⩾ 30	0.85	0.85	0.001

RESULTS

The results of the two-point analyses are summarized in Table 3. The lods for the Icelandic families have been summed to simplify the table. The highest lod score for any individual Icelandic family was 0.189; therefore, there was no indication of a suggestive positive lod being obscured by heterogeneity. No statistically significant evidence for linkage was found for any of the markers examined. The highest lod score was 1.63 at the locus D11S29 for Amish pedigree 110 alone at a recombination fraction of 0.10. The maximum lod for the combined Icelandic and Amish families at this locus was reduced to 1.42 also at $\theta = 0.1$ by negative lods in the Icelandic families. The Icelandic families were not very informative at this locus, yielding lods at $\theta = 0.10$ of 0.006, 0.016, and −0.235 for families 4001, 4002, and 4003, respectively. This mildly positive lod score is mitigated by strongly negative lod scores at the two nearby flanking markers, D11S144 and D11S147, which yielded lods of −3.70 and −3.03, respectively, for the Amish at $\theta = 0.0$. The D_2 dopamine receptor locus (DRD2) was excluded with a lod of −4.02 at $\theta = 0.0$. This is primarily due to the Amish family, as the Icelandic families were not very informative at this locus. For the other markers examined, linkage could be excluded for eight of the 13 loci at $\theta = 0.0$ using the combined family set. Lod scores for the other five loci were negative, but inconclusive.

Results of the multipoint analyses are displayed in

Table 3. Two-Point Lods for Bipolar Disorder versus Chromosome 11 Loci

	θ						
Locus	**0.00**	**0.01**	**0.05**	**0.10**	**0.20**	**0.30**	**0.40**
HRAS							
Icelandic	−0.43	−0.37	−0.22	−0.11	−0.02	0.00	0.01
Amish	−3.49	−3.10	−1.94	−0.96	−0.01	0.15	−0.03
Total	−3.93	−3.47	−2.15	−1.07	−0.03	0.15	−0.02
PTH							
Icelandic	−1.34	−1.09	−0.65	−0.41	−0.17	−0.06	−0.01
Amish	0.41	0.40	0.34	0.28	0.17	0.09	0.04
Total	−0.93	−0.69	−0.31	−0.13	0.00	0.03	0.03
D11S16							
Icelandic	0.02	0.01	0.01	0.00	−0.01	−0.01	−0.01
Amish	−1.83	−1.45	−0.66	−0.16	0.28	0.32	0.19
Total	−1.81	−1.44	−0.65	−0.16	0.27	0.31	0.18
CAT							
Icelandic	−1.10	−0.98	−0.67	−0.46	−0.22	−0.10	−0.03
Amish	−1.74	−1.03	−0.47	−0.28	−0.17	−0.11	−0.05
Total	−2.84	−2.01	−1.14	−0.74	−0.39	−0.21	−0.08
D11S288							
Icelandic	0.04	0.04	0.03	0.03	0.01	0.00	0.00
Amish	−1.42	−1.34	−1.05	−0.73	−0.28	−0.03	0.05
Total	−1.38	−1.30	−1.02	−0.70	−0.27	−0.03	0.05
D11S97							
Icelandic	−6.72	−5.51	−3.90	−2.65	−1.16	−0.42	−0.08
Amish	−8.13	−6.40	−3.99	−2.63	−1.20	−0.51	−0.15
Total	−14.85	−11.91	−7.89	−5.28	−2.36	−0.93	−0.23
D11S84							
Icelandic	−2.28	−2.08	−1.48	−0.99	−0.43	−0.16	−0.04
Amish	−4.17	−3.68	−2.49	−1.60	−0.63	−0.19	−0.02
Total	−6.45	−5.75	−3.97	−2.59	−1.06	−0.35	−0.07
DRD2							
Icelandic	−0.07	−0.07	−0.08	−0.07	−0.05	−0.03	−0.01
Amish	−3.95	−3.21	−1.96	−1.10	−0.23	0.03	−0.01
Total	−4.02	−3.29	−2.04	−1.17	−0.28	−0.01	−0.02
D11S144							
Icelandic	−0.02	−0.02	−0.03	−0.04	−0.03	−0.02	−0.01
Amish	−3.70	−3.41	−2.60	−1.92	−1.03	−0.46	−0.12
Total	−3.71	−3.43	−2.63	−1.96	−1.06	−0.48	−0.13
D11S29							
Icelandic	−0.41	−0.38	−0.30	−0.21	−0.11	−0.05	−0.01
Amish	0.30	1.09	1.58	1.63	1.35	0.85	0.30
Total	−0.11	0.71	1.29	1.42	1.24	0.81	0.29
D11S147							
Icelandic	−2.76	−2.00	−1.25	−0.84	−0.40	−0.16	−0.03
Amish	−3.03	−2.88	−2.29	−1.71	−0.94	−0.45	−0.15
Total	−5.78	−4.87	−3.54	−2.55	−1.34	−0.61	−0.19
D11S83							
Icelandic	0.02	0.02	0.01	0.01	0.00	0.00	0.00
Amish	−2.57	−1.82	−1.10	−0.72	−0.31	−0.10	−0.01
Total	−2.55	−1.80	−1.08	−0.71	−0.31	−0.10	−0.01
D11S419							
Icelandic	−0.52	−0.47	−0.29	−0.13	0.03	0.06	0.03
Amish	−0.43	−0.37	−0.20	−0.06	0.05	0.05	0.02
Total	−0.95	−0.84	−0.49	−0.19	0.08	0.11	0.04

Figure 2. The markers employed span a distance of 155 cM on chromosome 11. No positive lod scores were obtained in any of the analyses. The multipoint analyses exclude linkage for much of the region covered, except for an interval of approximately 40 cM on 11p. Several of the 11p markers examined had only limited informativeness in these families, thereby restricting our ability to examine this region of the chromosome. The region of D11S29 that was positive in the two-point analysis can be excluded in the multipoint analyses.

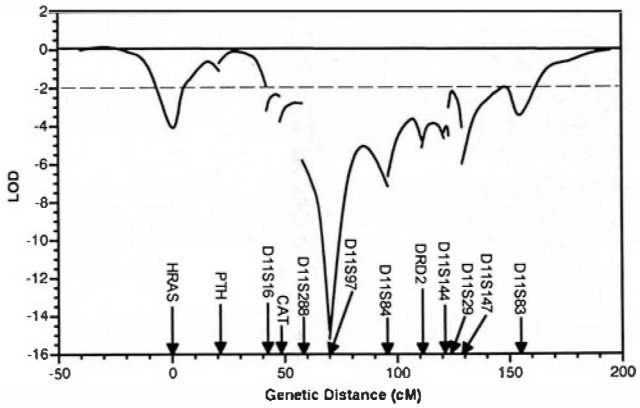

Figure 2. Multipoint analyses of bipolar disorder and chromosome 11 markers. Analyses include both Icelandic and Amish data. Each curve represents a separate three-point analysis moving the bipolar disorder locus through the interval between two markers. The dashed line at "LOD −2" represents the conventional criteria for exclusion of linkage.

This is consistent with the negative lod scores obtained from the two close flanking markers. The DRD2 is also excluded in this analysis.

DISCUSSION

In summary, no evidence was found for linkage to bipolar disorder for any of the chromosome 11 markers examined. Linkage of bipolar disorder to DRD2 is strongly excluded by these data. Mildly positive lod scores were obtained at D11S29, although strongly negative lods at nearby flanking markers argue against linkage in this region. Nevertheless, D11S29 is in the approximate region of the breakpoints for chromosomal translocations, which have been reported to segregate with bipolar disorder or psychotic disorder as described above. For this reason, this region deserves further study in a larger set of families.

These results are similar to those from several other studies of bipolar disorder and chromosome 11 markers. In particular, several other studies have examined the DRD2 locus and found no evidence for linkage. Byerley et al. (1990) examined four families from Utah and excluded linkage to DRD2 in three of these families. Holmes et al. (1991) examined five Icelandic families and excluded linkage to DRD2 and three other 11q markers: MS51, 2-14, and 6-3. Similarly, Mitchell et al. (1992) excluded linkage to DRD2 in two large Australian families, and Nöthen et al. (1992) found no evidence for involvement of DRD2 in an association study. As described above, the 11p15 region has been extensively studied, because of the previous report of linkage to the markers HRAS and INS in the Amish (Egeland et al. 1987). The data for HRAS in the Amish, presented in this study, represent the HRAS genotypes presented previously (Kelsoe et al. 1989), but they have been reanalyzed with diagnostic changes. These changes result from two previously unaffected individuals who have had recent onsets of illness and a somewhat more stringent diagnostic scheme in which those with a single episode of major depression are considered unknown instead of affected (see Methods). This reanalysis results in similar conclusions, although the lod scores are not as strongly negative. For HRAS at $\theta = 0$, the previous lod was −9.31, whereas in this analysis, the lod was −3.49. The Icelandic families are relatively uninformative for HRAS, and the lod scores are inconclusive.

Pakstis et al. (1991) have recently reported a survey of 185 markers that they have examined in an 81-member subset of Amish pedigree 110. Their study includes several of the chromosome 11 probes that we have studied in the expanded pedigree. In general, our results agree well with theirs, although, the lods of the larger pedigree are more negative. They obtained a maximum lod of 1.19 for HRAS at $\theta = 0.2$ and a minimum lod of −1.72 at $\theta = 0$. Our results are more negative with a lod of −3.49 in the Amish at $\theta = 0$ and a maximum lod of only 0.15 at $\theta = 0.3$. This is consistent with previous studies that indicated that the 31-member right extension to pedigree 110 yields strongly negative lods for HRAS and contributed to the overall exclusion in the expanded pedigree (Kelsoe et al. 1989). The DRD2 locus yielded a negative lod of only −0.89 at $\theta = 0$ in their data, whereas it is clearly excluded in our data with a lod of −3.95 at $\theta = 0$. In their study, D11S29 yielded a maximum lod of 1.58 at $\theta = 0$, which is similar to the maximum lod of 1.63 that we obtained at $\theta = 0.1$.

These results must be qualified by their dependence on the assumed mode of transmission. Because of this, it is difficult in linkage studies to "exclude" a region with any degree of certainty. The model of transmission employed is derived from a segregation analysis of Amish families. As described above, we did not believe that it was possible to conduct a similar segregation analysis in the Icelandic families, and, therefore, we used the model from the Amish. Although we believe that this is a reasonable assumption, it is nevertheless a limitation of this study. Another assumption in these analyses is that of genetic homogeneity. It is likely that bipolar disorder is heterogeneous, and, therefore, it is possible that there is more than one gene for bipolar disorder in these families. In fact, some historical data suggest the possibility of more than one gene for bipolar disorder in the Amish pedigree (Kelsoe et al. 1989). It is difficult to analyze these data under an assumption of heterogeneity because of the small number of families and the computational demands of conducting a two-locus analysis in a pedigree as large as the Amish. Nevertheless, simulation studies have indicated that under many conditions, two-point analyses are relatively robust to both misspecification of the mode of

transmission and heterogeneity (Cox et al. 1988; Vieland et al. 1992), although the effect of errors in both of these factors is less clear. Multipoint analyses may not be so robust, and, therefore, those results should be more cautiously considered (Risch and Giuffra 1990). We are continuing to survey the genome in these families both by systematic mapping and testing of specific candidate genes in search of a locus for bipolar disorder.

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REFERENCES

Anatonarakis SE, Phillips JA, Mallonee RL, Kazazian HH, Fearon ER, Waber PG, Kronenberg HM, Ullrich A, Meyers DA (1983): Beta-globin locus is linked to the parathyroid hormone locus (PTH) and lies between the insulin and PTH loci in man. Proc Natl Acad Sci (USA) 80:6615–6619

Bonner TI, Modi WS, Seuanez HN, O'Brien SJ (1991): Chromosomal mapping of five human genes encoding muscarinic acetylcholine receptors. Cytogenet Cell Genet 58:1850–1851

Byerley W, Plaetke R, Hoff M, Jensen S, Holik J, Reimherr F, Mellon C, Wender P, O'Connell P, Leppert M (1992): Tyrosine hydroxylase gene not linked to manic-depression in seven of eight pedigrees. Hum Hered 42:259–263

Byerley WF, Leppert M, O'Connell P, Mellon C, Holik J, Lubbers A, Reimherr F, Jenson S, Hill K, Wender P, Grandy D, Litt M, Lalouel J-M, Civelli O, White R (1990): D2 dopamine receptor gene not linked to manic-depression in three families. Psychiatr Genet 1:55–62

Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV (1983): Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature 302:33–37

Craig SP, Buckle VJ, Lamouroux A, Mallet J, Craig I (1986): Localization of the human tyrosine hydroxylase gene to 11p15: Gene duplication and evolution of metabolic pathways. Cytogenet Cell Genet 42:29–32

Craig SP, Boularand S, Darmon MC, Mallet J, Craig IW (1991): Localization of human tryptophan hydroxylase (TPH) to chromosome 11p15.3–p14 by in situ hybridization. Cytogenet Cell Genet 56:157–159

Cox NJ, Hodge SE, Marazita ML, Spence MA, Kidd KK (1988): Some effects of selection strategies on linkage analysis. Genet Epidemiol 5:289–297

Detera-Wadleigh SD, Berrettini WH, Goldin LR, Boorman D, Anderson S, Gershon ES (1987): Close linkage of c-Harvey-ras-1 and the insulin gene to affective disorder is ruled out in three North American pedigrees. Nature 325:806–808

Egeland JA, Hostetter AM (1983): Amish Study, I: Affective disorders among the Amish, 1976–1980. Am J Psychiatry 140:56–61

Egeland JA, Gerhard DS, Pauls DL, Sussex JN, Kidd KK, Allen CR, Hostetter AM, Housman DE (1987): Bipolar affective disorders linked to DNA markers on chromosome 11. Nature 325:783–787

Endicott J, Spitzer R (1978): A diagnostic interview: The Schedule for Affective Disorders and Schizophrenia. Arch Gen Psychiatry 35:837–844

Feder J, Yen L, Wijsman E, Wang L, Wilkens L, Schroder J, Spurr N, Cann H, Blumenberg M, Cavalli-Sforza LL (1985): A systematic approach to detecting high-frequency restriction fragment polymorphisms using large genomic probes. Am J Hum Genet 37:635–649

Feinberg AP, Vogelstein B (1983): A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gelernter J, Kennedy JL, Van Tol HH, Niznik HB, Civelli O, Kidd KK (1991): The D4 dopamine receptor (DRD4) maps to distal 11p close to HRAS. Cytogenet Cell Genet 58:1960

Gill M, McKeon P, Humphries P (1988): Linkage analysis of manic depression in an Irish family using H-ras 1 and INS. J Med Genet 25:634–65

Grandy DK, Litt M, Allen L, Bunzow JR, Marchionni MA, Makam H, Reed L, Magenis RE, Civelli O (1989): The human dopamine D2 receptor gene is located on chromosome 11 at q22–23 and identifies a TaqI RFLP. Am J Hum Genet 45:778–785

Grewal RP, Martinez M, Hoehe M, Bonner TI, Gershon ES, Detera-Wadleigh S (1992): Genetic linkage mapping of the m4 human muscarinic receptor (CHRM4). Genomics 13:239–240

Helgason T (1979): Epidemeological investigations concerning affective disorders. In Schou M, Stromgren E (eds), Origin, Prevention and Treatment of Affective Disorders. London, Academic Press, pp 241–256

Hodgkinson S, Sherrington R, Gurling H, Marchbanks R, Reeders S, Mallet J, McInnis M, Petursson H, Brynjolfsson J (1987): Molecular genetic evidence for heterogeneity in manic depression. Nature 325:805–86

Holland T, Gosden C (1990): A balanced chromosomal translocation partially co-segregating with psychotic illness in a family. Psychiatry Res 32:1–8

Holmes D, Brynjolfsson J, Brett P, Curtis D, Petursson H, Sherrington R, Gurling H (1991): No evidence for a susceptibility locus predisposing to manic depression in the region of the dopamine (D2) receptor gene. Br J Psychiatry 158:635–641

Hostetter AM, Egeland JA, Endicott J (1983): Amish Study.

II: Consensus diagnosis and reliability results. Am J Psychiatry 140:62–66

Jeffreys AJ, Wilson V, Neumann R, Keyte J (1988): Amplification of human minisatelites by the polymerase chain reaction: Towards DNA fingerprinting of single cells. Nucl Acids Res 16:10953–10971

Jimerson DC (1987): Role of dopamine mechanisms in the affective disorders. In Meltzer HY (ed), Psychopharmacology: The Third Generation of Progress. New York, Raven, pp 505–511

Julier C, Nakamura Y, Lathrop GM, O'Connell P, Leppert M, Litt M, Mohandas T, Lalouel JM, White R (1990): A detailed genetic map of the long arm of chromosome 11. Genomics 7:335–345

Junien C, van Heyningen V (1990): Report of the Committee on the Genetic Constitution of chromosome 11. Cytogenet Cell Genet 55:153–169

Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, Long RT, Kidd KK, Conte G, Housman DE, Paul SM (1989): Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. Nature 342:238–243.

Lathrop GM, Lalouel JM, Julier C, Ott J (1984): Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci (USA) 81:3443–3446

Law A, Richard CW III, Cottingham RW Jr, Lathrop GM, Cox DR, Myers RM (1992): Genetic linkage analysis of bipolar affective disorder in an Old Order Amish pedigree. Hum Genet 88:562–568

Litt M, White RL (1985): A highly polymorphic locus in human DNA revealed by cosmid-derived probes. Proc Natl Acad Sci (USA) 82:6206–6210

Maslen C, Jones C, Glaser T, Magenis RE, Sheehy R, Kellog J, Litt M (1988): Seven polymorphic loci mapping to human chromosomal region 11q22-qter. Genomics 2:66–75

Mendlewicz J, Leboyer M, De Bruyn A, Malafosse A, Sevy S, Hirsch D, Van Broeckhoven C, Mallett J (1991): Absence of linkage between chromosome 11p15 markers and manic-depressive illness in a Belgian pedigree. Am J Psychiatry 148:1683–1687

Mitchell P, Waters B, Morrison N, Shine J, Donald J, Eisman J (1991): Close linkage of bipolar disorder to chromosome 11 markers is excluded in two large Australian pedigrees. J Affect Disord 21:23–32

Mitchell P, Selbie L, Waters B, Donald J, Vivero C, Tully M, Shine J (1992): Exclusion of close linkage of bipolar disorder to dopamine D1 and D2 receptor gene markers. J Affect Disord 25:1–11

Nanko S, Kobayashi M, Gamou S, Kudoh J, Shimizu N, Takazawa N, Kazamatsuri H, Furusho T (1991): Linkage analysis of affective disorder using DNA markers on chromosomes 11 and X. Jpn J Psychiatry Neurol 45:53–56

NIGMS (1992): Catalog of cell lines: NIGMS human genetic mutant cell repository. Bethesda, NIH

Nöthen MM, Erdmann J, Korner J, Lanczik M, Fritze J, Fimmers R, Grandy DK, O'Dowd B, Propper P (1992): Lack of association between dopamine D1 and D2 receptor genes and bipolar affective disorder. Am J Psychiatry 149:199–201

Owen M, Parfitt E, Sargeant M, Asherson P, McGuffin P, Taylor C, Collier D, Powell J, Murray R, Gill M (1991): Linkage studies of schizophrenia with markers of chromosome 11q. Am J Hum Genet 49 (Suppl):354

Pakstis AJ, Kidd JR, Castiglione CM, Kidd KK (1991): Status of the search for a major genetic locus for affective disorder in the Old Order Amish. Hum Genet 87:475–483

Pauls DL, Gerhard DS, Lacy LG, Hostetter AM, Allen CR, Bland SD, LaBuda MC, Egeland JA (1991): Linkage of bipolar affective disorders to markers on chromosome 11p is excluded in a second lateral extension of Amish pedigree 110. Genomics 11:730–736

Quan F, Korneluk RG, MacLeod HL, Tsui LC, Gravel RA (1985): An RFLP associated with the human catalase gene. Nucl Acids Res 13:8288 (Abstract)

Rice J, Reich T, Andreasen NC, Endicott J, van Eerdewegh M, Fishman R, Hirschfeld RM, Klerman GL (1987): The familial transmission of bipolar illness. Arch Gen Psychiatry 44:441–447

Risch N, Giuffra L (1990): Multipoint linkage analysis of genetically complex traits. Am J Hum Genet 47:A197 (Abstract)

Sambrook J, Fritsch EF, Maniatis T (1989): Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press

Sanders AR, Hamilton JD, Fann WE, Patel PI (1991): Schizophrenia candidate gene association study. Am Psychiatr Assoc Abstr 144:49

Smith M, Wasmuth J, McPherson JD, et al (1989): Cosegregation of an 11q22.3-9p22 translocation with affective disorder: Proximity of the dopamine D2 receptor gene relative to the translocation breakpoint. Am J Hum Genet 45:A220 (Abstract)

Spitzer R, Endicott J, Robins E (1978): Research diagnostic criteria: Rationale and reliability. Arch Gen Psychiatry 35:773–782

St. Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, Gosden C, Evans HJ (1990): Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336:13–16

Su Y, Bray J, Burke J, Inomata T, MacLean C, Marazita M, Murphy B, Nie L, Nuallain MN, O'Neil A, Ploughman L, Shinkwin R, Walsh D, Keldler K, Diehl S (1991): Linkage studies of schizophrenia: Exclusion of candidate regions on chromosomes 5q and 11q. Am J Hum Genet 49 (Suppl):361

Turner E, Ewing J, Shilling P, Smith TL, Irwin M, Schuckit M, Kelsoe JR (1991): Lack of association between an RFLP near the D2 dopamine receptor gene and severe alcoholism. Biol Psychiatry 31:285–290

Vieland VJ, Hodge SE, Greenberg DA (1992): Adequacy of single-locus approximations for linkage analyses of oligogenic traits. Genet Epidemiol 9:45–59

Warnich L, Kotze MJ, Retief AE, Dietzsch E, Fox MF, Kotze GM, Nicholson DL, Retief E, Oosthuizen CJJ (1986): An anonymous human single copy genomic clone, D11S29 (L7) at 11q23, identifies a moderately frequent RFLP. Nucl Acids Res 14:1920 (Abstract)

Weber JL, May PE (1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396