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Genetics

Chromosome 18 DNA markers and manic–depressive illness: Evidence for a susceptibility gene

(bipolar illness/linkage/genetic vulnerability)

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ABSTRACT **In the course of a systematic genomic survey, 22 manic–depressive (bipolar) families were examined for linkage to 11 chromosome 18 pericentromeric marker loci, under dominant and recessive models. Overall logarithm of odds score analysis for the pedigree series was not significant under either model, but several families yielded logarithm of odds scores consistent with linkage under dominant or recessive models. Affected sibling pair analysis of these data yielded evidence for linkage ($P < 0.001$) at *D18S21*. Affected pedigree member analysis also suggests linkage, with multilocus results for five loci giving $P < 0.0001$ and $P = 0.0007$ for weighting functions $f(p) = 1$ and $1/\sqrt{p}$, respectively, where p is the allele frequency. These results imply a susceptibility gene in the pericentromeric region of chromosome 18, with a complex mode of inheritance. Two plausible candidate genes, a corticotropin receptor and the α subunit of a GTP binding protein, have been localized to this region.**

Evidence from twin, family, and adoption studies suggests that bipolar (BP) illness is, in part, a heritable disease (1). Linkage studies of BP disorder have been conducted to localize causative genes; however, no confirmed linkages have been established. Evidence for a BP gene on 11p15 (2) has been discounted by failure to replicate the finding in numerous other pedigrees (3–6) and by evaluation of newly ascertained individuals in the original pedigree (7). The color-vision region of Xq28 also has been reported linked to BP illness (8–11). Again, however, several investigators have not confirmed this linkage (12–15). One (11) of the most positive original findings [logarithm of odds (lod) score of 9 in four Israeli pedigrees for clinically assessed color blindness and glucose-6-phosphate dehydrogenase deficiency] could not be replicated by molecular genotyping with relevant Xq28 DNA markers (16).

It has been suggested that failures of linkage replication may reflect statistical artifacts of multiple testing, false rejection of linkage due to misspecification of the genetic model, and low statistical power to detect linkage (17). Some nonparametric analytic approaches, including affected sibling pair [ASP (18)] and affected pedigree member [APM (19)] methods, do not require specification of the genetic model of illness inheritance and, thus, may be appropriate for complex diseases (see below).

We have been conducting a genomic search for BP vulnerability genes, using a series of multiplex BP families (20–22). We report here results for analysis of several pericentromeric chromosome 18 DNA markers (23) that provide preliminary evidence for a susceptibility gene in this region under nonparametric methods.

METHODS

Clinical Description. Of the 22 families studied, we have previously described (20) 21 pedigrees. These families consist of 365 individuals, 159 of whom are ill. Diagnoses were based on personal semistructured interviews, medical records, and information from relatives. To this series, the "right" extension of the Old Order Amish pedigree 110 (7) was added as the 22nd pedigree. The diagnostic methods for this pedigree (2, 7) are similar to those employed for the remaining 21 pedigrees and have been corroborated by careful review of case notes and narratives kindly provided by J. A. Egeland (personal communication). This branch of the Old Order Amish pedigree 110, designated pedigree 9000 in the tables, contains 30 informative individuals, 8 of whom are affected. This branch was chosen because it appeared from the published pedigree that there was a single known ancestor with BP illness within this branch. We thought it prudent not to study other branches of this large pedigree because the risk of intrapedigree genetic heterogeneity appeared greater.

Two definitions of affection status were used. Affection status model I included schizoaffective, BPI, and BPII with major depression and model II included recurrent unipolar illness in addition to those diagnoses mentioned above. These two definitions of the BP spectrum are supported by twin, family, and adoption studies, as discussed elsewhere (20).

Genetic Marker Typing. DNA was extracted from immortalized lymphoblastoid cell lines or from peripheral blood samples (24). Primers for the *D18S37, D18S40, D18S44, D18S45* (25), *D18S453* (described in the Genome Data Base), and *D18S56, D18S66, D18S62,* and *D18S53* (26) loci were purchased from Research Genetics (Huntsville, Alabama). Microsatellite genotyping was done as described (21). Briefly, one primer was end-labeled with [γ-^{32}P]ATP (New England Nuclear) by using T4 polynucleotide kinase. A 20-μl polymerase chain reaction (PCR) volume included genomic DNA (30 ng), 200 μM dATP, 200 μM dGTP, 200 μM dCTP, and 200 μM TTP, 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 2 pM labeled primer, 5 pM unlabeled primer, 50 μM tetramethylammonium chloride, 0.001% gelatin, 2.5 mM $MgCl_2$, and 0.5 unit of *Taq* polymerase. Genotypes were read by two readers and matched by computer. Discrepancies were resolved by reference to a third reader. Readers had no knowledge of the diagnosis of a given individual.

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Abbreviations: BP, bipolar; ACTH, corticotropin; APM, affected pedigree member; ASP, affected sibling pair; lod, logarithm of odds; cM, centimorgan(s).

A minisatellite probe (cMS615) for locus *D18S32* was a gift from John Armour (University of Leiceister, U.K.). Although it has not been placed on any published linkage map, preliminary evidence placed it in this region (27). Genotyping methods for *D18S32* (using Southern blots of genomic DNA digested with *Hinf*I) and *D18S21* (probe pHHH163) have been described (21).

Linkage Analyses. lod score calculations, using the LINKAGE program (version 5.10), were done assuming a maximum (age-dependent) penetrance of 50% (and 85%; results not presented) for an autosomal dominant model, with a 2.5% disease allele frequency. An age-dependent penetrance of the normal genotype ranged from 0.1 to 1% to allow for a small number of phenocopies. Calculations assuming a recessive inheritance with 85% penetrance (15% disease allele frequency) were also done. Tests of heterogeneity were done with the HOMOG program (28).

Nonparametric methods were also applied to these data. We computed the ASP statistics that test whether ASPs have a mean proportion of marker genes identical-by-descent that is >0.50 (18). The sib-pair statistics were computed (using all of the available data on the sibship and their parents) by using the SIBPAL program of the SAGE package (29). These statistics are computed on all possible affected pairs. This procedure may inflate the contribution of sibships with large numbers of affected individuals. To partially correct for this, we modified the degrees of freedom of the t test to be the number of independent affected pairs (defined per sibship as the number of affected individuals minus 1) in the sample instead of the number of all possible pairs (30).

An APM analysis was applied to these data (19, 31). These methods use marker information of affected individuals to test whether the affected persons within a pedigree are more similar to each other at the marker locus than would be expected by chance. The marker similarity is measured in terms of identity-by-state. The APM method uses a marker allele frequency weighting function, $f(p)$, where p is the allele frequency, and the APM test statistics are presented separately for each of three different weighting functions, $f(p) = 1$, $f(p) = 1/\sqrt{(p)}$, and $f(p) = 1/p$. Whereas the second and third functions render the sharing of a rare allele among affected persons a more significant event, the first weighting function uses the allele frequencies only in calculation of the expected degree of marker allele sharing. The third function, $f(p) = 1/p$, may lead (more frequently than the first two) to a non-normal distribution of the test statistic. The second function, $f(p) = 1/\sqrt{(p)}$, is a reasonable compromise for generating a normal distribution of the test statistic while incorporating an allele frequency function. The APM test statistics are sensitive to marker locus allele frequency misspecification (32). These allele frequencies were estimated from the studied pedigrees by using the method of Boehnke (33) or by counting alleles.

Significance of the APM test statistic is calculated from the theoretical (normal) distribution of the statistic. In addition, 10,000 replicates of these data, assuming independent inheritance of marker alleles and disease (i.e., no linkage), were simulated to assess the probability of observing the actual results (or a more extreme statistic) by chance. This probability is the empirical P value. Each replicate is generated by simulating an unlinked marker segregating through the actual pedigrees. An APM statistic is generated by analyzing the simulated data set exactly as the actual data set was analyzed. The rank of the observed statistic in the distribution of the simulated statistics determines the empirical P value.

A multilocus APM analysis was computed on these data (31). Some of the markers typed have been mapped in the CEPH (Centre d'Etude Polymorphism e Humain) data (23). We used the build option of the CRI-MAP program (34) on the genotypes in our families, with no predetermined marker locus order, to generate a more complete multipoint map. The map generated from our genotypes (1000:1 odds) agreed with the published order (23). The map order from our data [Kosambi centimorgan (cM), sex averaged] was Telomere—*D18S62*—15 cM—*D18S21*—10 cM—*D18S37*—2.6 cM—*D18S53*—1.9 cM—*D18S40*—3.6 cM—*D18S45*—4.1 cM—*D18S44*—10 cM—*D18S66*—3 cM—*D18S56*. The order of *D18S32* relative to *D18S40* could not be determined unambiguously. Similarly, a unique position of *D18S453* could not be determined between *D18S37* and *D18S40*.

Table 1. Summary of individual pedigree lod scores >1

Marker locus	Pedigree with dominant 50% penetrance	lod score	Pedigree with recessive 85% penetrance	lod score
D18S53	9000	1.25	1482	1.08
			48	2.38
D18S40	1442	1.09	1482	1.38
D18S45	9000	1.20		
D18S44			48	1.72
D18S32	1442	1.49	1520	1.84
D18S453			1482	1.20
			48	1.56

Genetic parameters are in text. Affection status model II with 1% recombination was used.

RESULTS

The lod score analysis of all 22 families, assuming homogeneity, yielded quite negative total lod scores for these chromosome 18 markers (two-point lod scores for all markers in individual families are available on request from the authors) regardless of the affection status model or the disease model. However, inspection of the two-point scores for individual families (Table 1) indicated that several kindreds yielded lod scores consistent with linkage based upon simulations assuming a dominant 50% penetrance model (20). Two-point linkage analysis assuming recessive transmission (with allele frequency of 15% and 85% penetrance) revealed positive lod scores in three other pedigrees (highest score, 2.38) as shown in Table 1. We also calculated lod scores in an "affected individuals only" analysis, and the results were similar to those using well individuals (data not shown). Analysis with the HOMOG program did not support heterogeneity under any tested model of disease inheritance.

We then considered that allelic heterogeneity or another complex mode of inheritance could have led to this mixture of families that were positive under different models. We performed model-free analyses, ASP and APM. Table 2

Table 2. ASP analysis of chromosome 18 markers

Marker	Proportion identical by descent	P value
D18S62	0.57	0.0016
D18S21	0.58	0.0004
D18S37	0.56	0.0083
D18S53	0.54	0.053
D18S453	0.54	0.153
D18S32	0.57	0.014
D18S40	0.57	0.012
D18S45	0.51	0.40
D18S44	0.48	1.00
D18S66	0.57	0.024
D18S56	0.57	0.0072

P values were adjusted for multiple sib-pairs within sibships by reducing the degrees of freedom in the t test to the number of independent comparisons ($r - 1$ for each sibship, where r is the number of affected sibs). Affection status model 2 was used.

shows the results of the ASP analyses for affection status model II. Seven of 11 markers have *P* values for allele sharing that are <0.05, with one marker (S21) having *P* value of <0.001. The two-point APM results do not show any markers to have highly significant sharing (data not shown). The multipoint APM analysis is suggestive for linkage with empirical *P* values of <0.0001 and 0.0007 for the weighting functions, $f(p) = 1$ and $f(p) = 1/\sqrt{p}$, respectively (Table 3).

DISCUSSION

Positive lod scores were observed for chromosome 18 pericentromeric markers under dominant or recessive models of inheritance for several pedigrees. However, significant evidence among all pedigrees for linkage and heterogeneity, using the lod score method, was not observed. The APM statistics (Table 3) provide evidence that a gene that increases susceptibility to BP illness is present in this region of chromosome 18. The ASP analysis is consistent with this hypothesis (Table 2).

It should be noted that the ASP statistic is computed on all possible pairs within a sibship. A simulation by Suarez and Van Eerdewegh (30) showed an increase in the type I error for an ASP statistic based on all possible pairs. However, when the statistic was weighted for sibship size, the type I error was generally not increased. In this study, we have corrected the degrees of freedom of the ASP tests to reflect the total number of independent affected pairs, but the statistics are not weighted by sibship size.

The fact that the nonparametric results have higher significance than the lod-score results may indicate complex inheritance (or small effect) of the susceptibility locus since the lod score method is not powerful for detecting genes with "small" effects (35). Whatever the origins of these results, this evidence for a BP susceptibility gene must be confirmed in an independent set of families.

The fact that positive lod scores at the same loci were observed in separate families for recessive or dominant models of inheritance raises the question of whether different mutations at the same locus could produce diseases segregating differently, as dominant or recessive. Different mutations in the growth hormone gene (*GH1*) have been described for two forms (one dominant and the other recessive) of familial growth hormone deficiency (36). Thus, there is evidence that different mutations in one gene may produce clinically similar diseases that appear to be transmitted in dominant or recessive modes.

Table 3. Multilocus APM analysis

Weighting function	Statistic	*P* value	Empirical *P* value
	Map: S62—S37—S53—S40		
$f(p) = 1$	1.48	0.0692	0.0670
$f(p) = 1/\sqrt{(p)}$	1.74	0.0404	0.0438
$f(p) = 1/p$	0.99	0.1598	0.1492
	Map: S40—S45—S44—S66—S56		
$f(p) = 1$	3.66	0.00013	<0.0001
$f(p) = 1/\sqrt{(p)}$	3.41	0.00033	0.0007
$f(p) = 1/p$	1.71	0.04335	0.0550

Multilocus APM analyses were done using a genetic map as specified in the text. Affection status model II was used. Results for model I (data not shown) are similar. The weighting functions were used to examine the effect of varying allele frequencies (p) on the results. The empirical *P* value is the probability of observing a simulated value as large or larger than the actual statistic by chance, as determined by calculating 10,000 replicates of an unlinked marker on these 22 pedigrees. These results are based on 127 affected individuals who were typed for all markers.

Another possible explanation for this observation of positive lod scores at the same locus under both recessive and dominant models is the influence of epistatic and environmental factors that, with a chromosome 18 pericentromeric locus, partially determine BP disease expression. Depending on the influence of such factors, the hypothetical chromosome 18 pericentromeric BP susceptibility gene may appear to act in either a recessive or dominant manner. In the presence of environmental and epistatic factors that increase the risk for BP disease, a single copy of a putative chromosome 18 BP susceptibility allele may be sufficient to produce illness, whereas, if these factors are less influential, two copies of a chromosome 18 pericentromeric BP susceptibility allele may be necessary for disease expression.

We must also admit the possibility that the significant results could be a false positive. On the other hand, if we have identified a locus with relatively "small" effects, it may be very difficult to replicate these findings. Suarez *et al.* (37) have shown that when a locus that is part of a system of additive loci is identified by linkage analysis hundreds of independent families may be necessary to replicate the finding.

While the evidence presented here is consistent with a BP susceptibility gene near the centromere of chromosome 18, the data do not permit accurate localization of a linked interval. Certainly within the region examined (which spans 50 cM), multiple candidate genes (most of which are unidentified) must exist.

Two candidate genes in this region deserve mention, the first being a gene for the α subunit of a GTP binding protein that (when associated with β and γ subunits) transduces neurotransmitter receptor activation into second messenger system changes within the neuron (38). Lithium (an effective treatment for BP illness) inhibits the function of α subunits by decreasing the affinity with which the α subunit binds GTP (39), and antidepressants modulate the expression of GTP binding protein genes in brain (40). A gene for the α subunit of a GTP binding protein (41) has been localized to the 18p11 region (42). Immunocytochemical and Northern blot hybridization experiments suggest that this α subunit, G_{olf}, may be expressed only in nasal epithelium and in limited regions of mammalian brain, including olfactory tract, bulb, and tubercle, nucleus accumbens, striatum, and substantia nigra (43).

The second candidate gene is a corticotropin (ACTH) receptor gene. Disturbances of hypothalamic–pituitary–adrenal axis function have often been described in patients with mood disorders. These abnormalities include hypercortisolemia (with loss of the normal circadian rhythm and failure to suppress plasma cortisol after dexamethasone administration), enlarged adrenal glands, elevated cerebrospinal fluid corticotropin-releasing factor levels, and a blunted ACTH response to exogenous administration of corticotropin-releasing factor (44). An ACTH receptor gene [*MC2* (45)] has been localized to this same region of chromosome 18p11.2 (46). Mutations in an ACTH receptor gene might lead to the disturbances of hypothalamic–pituitary–adrenal axis function noted in patients with mood disorders.

In summary, we present evidence that suggests that a chromosome 18 pericentromeric locus increases susceptibility to BP disease. Additional pedigree linkage studies of this region and association experiments with G_{olf} and ACTH receptor genes may be useful in delineating genetic vulnerability to BP disease.

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