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Segregation and Linkage Analyses in Families of Patients with Bipolar, Unipolar, and Schizoaffective Mood Disorders

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SUMMARY

Hypotheses of single major locus transmission (autosomal and X chromosome) of major affective disorder (i.e., bipolar, unipolar, and schizoaffective) are tested using the Elston-Stewart likelihood method of pedigree segregation analysis. The sample consists of families of varying size ascertained through patients treated at the National Institute of Mental Health in Bethesda, Maryland. We test hypotheses on subsamples of families according to: (1) diagnosis of proband (75 bipolar I, 22 bipolar II, 18 unipolar, and six schizoaffective); (2) extreme value of a biological trait in the proband ("low" monoamine oxidase, "low" cerebrospinal fluid serotonin metabolite 5-HIAA); and (3) positive response to lithium in the proband. We cannot find evidence for single major locus transmission of major affective disorder from segregation analysis in any subsample of family even when the diagnostic classification of ill phenotypes is widened to include possible affective "spectrum" diagnoses. In addition, linkage studies of 21 autosomal markers do not provide evidence for single major locus transmission of illness. The maximum lod score, found for 30 families at the *MNS* locus, was 1.39 at 20% recombination.

INTRODUCTION

The classic form of affective disorder is bipolar (BP) manic-depressive illness, with incapacitating episodes of both mania and depression [1]. Unipolar (UP) depression, in which patients have only depressive episodes, has been thought by some

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investigators to be generally a separate disorder from the BP form. Twin and family studies suggest that, at least in families of BP patients, the same underlying susceptibility can be expressed as either BP or UP illness [2]. In schizoaffective disorder (SA), the patient has depressive episodes with mood incongruent psychotic features, or manic or depressive episodes with psychotic features that persist even when the mood is substantially improved. Family studies suggest that SA illness is genetically related to UP and BP illness [2–4].

The increased prevalence of illness in relatives of patients from that in the population has been well established for some time [2], but no one specific genetic hypothesis has been found to be consistent with the majority of studies. Both multifactorial and single-major-locus hypotheses are consistent with most familial data if one analyzes simply the prevalence rates of illness in first-degree relatives [5–8]. Identification of major loci through linkage studies of affective disorder to either X-chromosome markers [9–16] or the *HLA* locus [17–21] have given conflicting results.

In the past few years, more powerful statistical models have become available to test specific genetic hypotheses taking into account variable age of onset of illness and nonrandom ascertainment of families. Elston and Stewart [22] developed a likelihood method for pedigree analysis that allows for multigenerational pedigree data. This method has been expanded further [23–25] and used to test single-major-locus hypotheses for affective disorder.

Bucher and Elston [26] and Bucher et al. [27] used this method to test hypotheses of single-locus transmission of affective illness in several sets of family study data collected from 1919 to 1971 in Europe and the United States. Even though the original investigators each came up with different conclusions with regard to the mode of genetic transmission, the results of segregation analyses were consistent across data sets, indicating that there was some form of vertical transmission of illness in families but not compatible with a single Mendelian locus. A single large pedigree with UP affective illness first described by Ashby and Crowe [28] was analyzed for single-locus segregation using the Elston-Stewart method by Crowe et al. [29]. The analysis could not distinguish a hypothesis of single-major-locus transmission from a purely nongenetic transmission.

Another way to identify underlying genetic factors in a disease is to find some biological trait that differs between ill persons and normals and is transmitted in families. For example, some (but not all) investigators have found BP patients to have lower levels of platelet monoamine oxidase (MAO) than normal individuals (see review in Gershon et al. [30]). However, in one family study [31], ill relatives of "low" MAO probands did not have "low" values. Low cerebrospinal fluid (CSF) 5-HIAA in patients has been associated with an increased morbidity in relatives [32]. Traits such as these may identify subgroups of families with a specific mode of genetic transmission and therefore support the existence of heterogeneity. Similarly, if it can be shown that illness in some but not other families is strongly linked to genetic marker traits, then genetic heterogeneity may be present.

During the past several years, we have systematically collected data on a large series of families of BP, UP, and SA patients, including several large multigenerational families. Here we examine single gene transmission of affective disorders by

applying the Elston-Stewart method of pedigree analysis to this sample of families of BP, UP, and SA patients and to three subsamples of BP families according to a finding of "low" MAO, "low" CSF-5HIAA, and positive response to lithium in the proband. In addition, we test for linkage of affective illness to a series of 21 segregating marker traits.

MATERIALS AND METHODS: CLINICAL

The selection of probands, examination of relatives, and diagnostic criteria are described in detail elsewhere [15, 16, 18, 33] and will be summarized here. Patients admitted to the inpatient wards and outpatient clinics for affective disorders of the National Institute of Mental Health (NIMH) were screened for this study without regard to family history. Normal controls were medical patients at the National Institutes of Health who had no history of a psychiatric disorder. The Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) interview [34] and Research Diagnostic Criteria (RDC) [35] were modified as described [33]. These were the instruments and criteria followed throughout the study for both probands and relatives. Interviews were diagnosed blindly by two investigators.

The proband, spouse, and all living first-degree relatives were directly interviewed, subject to availability. Second-degree and more distant relatives were examined when illness appeared to be segregating in that branch of the pedigree. Family history information was obtained from each interviewed person about his own first- and second-degree relatives. Medical records were obtained, whenever available, for all individuals known to be treated or hospitalized for a psychiatric disorder. Information and medical records were obtained for other, more distant living and dead relatives when there was an indication of a psychiatric disorder. The general goal was to examine all the descendants of the parents of the earliest known ill person in a pedigree except for branches that did not appear to be segregating for illness. In this manner, we were able to develop data for several large multigenerational pedigrees.

The final diagnoses on each individual were made from the interview diagnosis, information from relatives, and medical records diagnosis. Diagnoses were assigned in order of their onset in the person's life. For the genetic analyses in this study, one diagnosis was assigned to an individual according to their most severe lifetime diagnosis as defined in [4].

The probands in the entire study included 11 SA, 96 BP I, 34 BP II,* 31 UP, and 43 normal controls; 4,179 relatives (including spouses) of patients could be diagnosed, with 1,079) examined personally and the remainder from family history and medical records information.

Blood samples for determination of genetic markers were drawn from all individuals personally examined. Phenotypes for the following eight red cell antigen systems were determined: ABO, Rhesus (Rh), MNS, Kell (K), P, Duffy (Fy), Kidd (Jk), and Lewis (Le). ABH secretor status (Se) was determined indirectly, using the red cell Lewis phenotype results. Phenotypes were also determined using standard methods [36–38] for the following 17 red cell and serum systems: adenylate kinase (AK), adenosine deaminase (ADA), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase 1 (PGM_1), phosphoglucomutase 2 (PGM_2), acid phosphatase (ACP), galactose-1-phosphate uridyl transferase (GALT), glutamic-pyruvic transaminase (GPT), esterase D (ESD), haptoglobin (Hp), transferrin (Tf), group specific component (Gc), alpha 1 antitrypsin (Pi), pseudocholinesterase (E_1), third complement component (C3), amylase-2 (AMY_2) and glyoxalase 1 (GLO). PGM_2, Tf, Pi, and E_1 were not segregating.

* Bipolar illness is divided into two types: BP I have depressive and manic episodes; BP II have depressive and hypomanic (mild mania not requiring hospitalization) episodes.

MATERIALS AND METHODS: GENETIC MODELS

General Model

The Elston-Stewart likelihood method [22] of pedigree segregation analysis was used to estimate parameters and test hypotheses of single-locus autosomal and X-chromosome transmission of affective illness.

The model assumes that the phenotype of illness is controlled by a single two-allele (*a, A*) locus, and there are three types of individuals denoted *aa, aA*, and *AA*. The population proportions of the three types are ψ_{aa}, ψ_{aA}, and ψ_{AA}. Under Hardy-Weinberg equilibrium assumptions, these proportions are a function of the allele frequency q_a. Persons of the three genotypes transmit allele *a* to their offspring with probabilities τ_{aaa}, τ_{aAa}, and τ_{AAa}, respectively, which under a simple Mendelian hypothesis are 1, ½, and 0.

We allow for three phenotypes: Z = 0, 1, 2, in order for individuals to be classified as normal, mildly affected, and severely affected, respectively.

The model includes parameters for penetrance $g_u(z)$, which are defined as the probability that an individual of genotype *u* has phenotype *z*. The particular form of the age-dependent penetrance function to be used in this study has been described [39]. In this function, the age-of-onset distributions are dependent on phenotype and susceptibility is dependent on genotype (i.e., μ_1, μ_2, and σ^2 represent the respective means and common variance for "mild" and "severe" illness). Susceptibility is defined as: $\gamma_u(z)$ = probability that an individual of genotype *u* has phenotype *z* provided he lives long enough. Ascertainment probability was assumed to be a constant parameter for these analyses.

Under a Mendelian hypothesis, the τ's are restricted to be 1, ½, and 0. If the ill phenotypes are dominant to the normal phenotypes, then $\gamma_{aa}(z) = \gamma_{aA}(z)$. Under a nongenetic or "environmental" hypothesis, there is no parent-offspring transmission and $\tau_{aaa} = \tau_{aAa} = \tau_{AAa}$.

These hypotheses are each tested against the general alternative hypothesis of arbitrary transmission probabilities ("unrestricted" hypothesis), using the likelihood ratio criterion (i.e., twice the difference in $\log_e$ likelihoods between the restricted and unrestricted hypotheses is distributed as a chi-square variable with degrees of freedom equal to the number of additional parameter restrictions imposed on the unrestricted hypothesis). Thus, all Mendelian hypotheses have 3 degrees of freedom (df), and the "environmental" hypotheses have 2 df. The program GENPED [40] was used to estimate parameters and compute the likelihood under the unrestricted model and under each hypothesis.

Phenotypic Models for Affective Disorder

Given the uncertainty as to which clinical diagnoses are manifestations of the same underlying susceptibility, we tested hypotheses and estimated parameters under several phenotypic models. These are outlined in table 1 and proceed from the most-restrictive to the least-restrictive models of illness based on several previous hypotheses. For instance, unexplained suicide may be an indication of a major affective disorder when there was not enough information to determine a psychiatric diagnosis preceding the suicide. As expected, this is found more often in distant relatives of the proband than in the first-degree relatives. A previous study by Gershon et al. [41] found that individuals with an undiagnosed major psychiatric disorder (in this study called "undiagnosed functional psychosis" and "other psychiatric disorder with hospitalization") occurred more often in families of affective disorder patients than in control families. However, the present study does not find this excess [4]. Several investigators find that "cyclothymic-personality" is a mild manifestation of BP illness [42]. The third classification scheme includes these additional diagnoses. Winokur [43] hypothesized that alcoholism and sociopathy are part of the depression "spectrum" in some families, and these are included (along with drug abuse) as "ill" in the fourth classification scheme. However, these particular diagnoses are not found more frequently in patient families than in control families in this study [4].

TABLE 1

PHENOTYPIC MODELS FOR AFFECTIVE ILLNESS

CLASSIFICATION MODEL NO.	PHENOTYPE 2	1	0
1	SA, BP I, BP II	UP (with incapacitation)	Other
2	Same as 1	All UP	Other
3	Same as 1 + suicide, other psychiatric disorder with hospitalization, undiagnosed functional psychosis	All UP + cyclothymic personality	Other
4	Same as 3 + UP	Cyclothymic personality, alcoholism, drug abuse, sociopathy	Other

Samples of Pedigrees for Segregation Analysis

Pedigrees were included in the segregation analysis when at least five persons were personally examined. Thus, a large subset of the original sample (121 out of 172) was included. The average pedigree size was 23. To minimize heterogeneity of the illness, we first analyzed families separately according to diagnosis of proband. In addition, we analyzed subsamples of BP pedigrees on the basis of extreme values of a biological trait in the proband as discussed earlier. We chose pedigrees in which the proband was in the lower 25% of the distribution of platelet MAO in probands (MAO less than 8.0 nmol/10^8 platelets/hr) and CSF 5-HIAA in probands (baseline CSF 5-HIAA less than 15 ng/ml, data from Post, personal communication, 1979).

We were also able to select pedigrees in which the proband was known to be a lithium responder (data from Nurnberger et al. [44]). The number of probands in these last three categories is small because of the amount of data available for each of these measurements.

Linkage Analysis

Lod scores for linkage of affective disorder to a series of marker traits was calculated using the LIPED program [45] with correction for age of onset. Heterogeneity of linkage was examined by using the test statistic given by Morton [46].

RESULTS

Preliminary Analyses

The distribution of age of onset of illness in our patient and relative sample was examined in order to determine the form of the correction to be used in the genetic model. Age of onset in this sample can be approximated by a $\log_e$ normal distribution. This is also a convenient distribution to use in the genetic likelihood function. There is no male-female difference in age of onset and no difference between probands and other ill relatives. Since BP individuals have a significantly lower age of onset than UP (27 years vs. 29 years), we allow for separate mean ages of onset for the "mild" and "severe" illness categories in the genetic model. The age-of-onset parameters are estimated for the sample of BP I families and then fixed in the other samples due to sample-size constraints. Because probands have a similar age-of-onset distribution as the ill relatives, the probability of ascertainment will be assumed to be a constant parameter.

In [4], we present age-corrected morbidity risk estimates for first-degree relatives of probands (total of 172 probands and 989 first-degree relatives). This information is condensed in table 2. In our sample, the age-corrected risk of major affective illness ranges from approximately 20% in UP families to 38% in SA families. In contrast, the risk in relatives of normal controls is approximately 7%. There are no significant differences in the proportion of ill male and ill female relatives and no difference in the proportion of ill relatives according to the sex of the proband. While most studies find a higher proportion of females than males for UP illness, this is not always the case. One current study of affective disorders in the Old Order Amish does not find a sex difference in the prevalence of UP illness either in the population or in the relatives of BP probands (J. Egeland, personal communication, 1982). This suggests that sex differences may be culturally determined.

Segregation Analysis

As described in MATERIALS AND METHODS: GENETIC MODELS, segregation analyses were performed in seven samples of pedigrees, using four different phenotypic classification models (table 1). We cannot find evidence for either a single autosomal or a single X-chromosome locus that accounts for the transmission of affective disorder in any of the samples of families analyzed according to diagnosis of proband (i.e., BP, UP, and SA) or in any of the subsamples of families chosen on the basis of a biological trait in the proband. This is true even when the classification of diagnoses of relatives into ill-well phenotypes is altered from the most restrictive to the least restrictive as described in table 1. An example of the hypothesis testing results for all the samples of pedigrees is shown in table 3, and an example of parameter estimates for the BP I families is shown in table 4. In this particular example, both single gene and environmental hypotheses tend to be strongly rejected. In one case (SA families), the X-chromosome and environmental hypotheses are not rejected and the autosomal hypothesis is rejected. Thus we cannot conclude that there is X-chromosome transmission. For UP families, genetic hypotheses are rejected but the environmental hypothesis is not rejected.

The other phenotypic models give essentially the same results. In addition, if the diagnoses—"undiagnosed functional psychosis" and "other psychiatric disorder

TABLE 2

LIFETIME PREVALENCES OF MAJOR AFFECTIVE DIAGNOSES IN FIRST-DEGREE RELATIVES OF PROBANDS (FROM GERSHON ET AL. [4])

	TYPE OF PROBAND (NO.)				
DIAGNOSIS OF RELATIVE	SA (11)	BP I (96)	BP II (34)	UP (31)	Control (43)
SA	6.1	1.1	0.6	0.7	0.5
BP I	10.7	4.5	2.6	1.5	0.0
BP II	6.1	4.1	4.5	1.5	0.5
UP	14.5	14.0	17.3	16.6	5.8
Total major affective	38.4	23.7	25.0	20.3	6.8

NOTE: Lifetime prevalences were corrected for age as described by Gershon et al. [4].

TABLE 3

CHI-SQUARE VALUES FOR TESTS OF HYPOTHESES FOR PHENOTYPIC CLASSIFICATION NO. 3

	No. families	Autosomal (3 df)	X chromosome (3 df)	Environmental (2 df)
Sample:				
SA	6	14.52	5.78*	1.78*
BP I	75	118.96	105.04	22.70
BP II	22	48.92	51.38	9.96
UP	18	25.18	18.8	4.82*
Bipolar subsamples:				
"Low" MAO	20	68.54	68.64	20.74
"Low" 5-HIAA	7	19.85	15.13	13.50
Lithium responders	18	30.62	19.16	12.00

NOTE: Phenotypic classification no. 3 is described in table 1.
* Hypothesis *not* rejected.

with hospitalization"—are removed* from the third model, the results of segregation analysis are unchanged.

Several generalizations can be made from these results. When the sample size is small, both genetic and environmental hypotheses are usually consistent with the data and have similar likelihoods. In most of the larger samples, all hypotheses tend to be rejected. The Mendelian hypotheses have such a poor fit that it is meaningless to differentiate between dominant and recessive hypotheses. As seen in table 4, under Mendelian transmission, the three genotypic susceptibilities, $\gamma_{aa}(2)$, $\gamma_{aA}(2)$, and $\gamma_{AA}(2)$, have nearly the same value, indicating that the data are not compatible with some dichotomy of genotype susceptibilities at a single locus. The fact that all of the genetic hypotheses tend to have similar likelihoods suggests that the model is inadequate for these data. The value of τ_{aaA} under the unrestricted hypothesis is usually much higher than zero, indicating transmission of illness from individuals without genetic susceptibility. For these reasons, it is not surprising that even though the environmental hypothesis tends to be rejected it usually has a much higher likelihood than do the genetic hypotheses.

Linkage Analysis

Despite the fact that segregation analyses do not support the existence of a general single major locus controlling affective disorders, it is possible that there is heterogeneity with some small proportion of families transmitting the illness as a single-locus trait. Table 5 shows lod scores for linkage of affective disorders (assuming dominant transmission) to 21 autosomal markers under phenotype model no. 3. The wider and narrower classifications of phenotypes do not greatly change the overall results. The highest lod score is 1.39 at $\theta = 20\%$ for the *MNS* locus in 30 segregating families. This is slightly suggestive of loose linkage. No subsample of pedigrees (as described in table 3) gives significantly higher lod scores for the *MNS* locus, and there is no overall heterogeneity of linkage by Morton's test in the 30 families ($\chi^2_{29} = 26.0$). The MNS scores are approximately the same for the

* It is unclear from family study data whether these diagnoses are part of an "affective spectrum."

TABLE 4

MAXIMUM-LIKELIHOOD ESTIMATES FROM A SAMPLE OF 75 BP I FAMILIES—PHENOTYPIC CLASSIFICATION NO. 3

PARAMETER	HYPOTHESIS				
	Autosomal	Environmental	Unrestricted	X chromosome	X chromosome unrestricted
τ_{aaa}	1	0.353	1.0	1	1.0
τ_{Aaa}	1/2	0.353	0.923	1/2	0.920
τ_{AAa}	0	0.353	0.401	0	0.580
q_a	0.725	0.075	0.092	0.476	0.195
$\gamma_{aa}(1)$	0.194	0.201	0.158	0.179	0.153
$\gamma_{Aa}(1)$	0.0	0.201	0.158	0.179	0.153
$\gamma_{AA}(1)$	0.0	0.037	0.043	0.0	0.024
$\gamma_{aa}(2)$	0.290	0.752	0.570	0.314	0.515
$\gamma_{Aa}(2)$	0.371	0.752	0.570	0.314	0.515
$\gamma_{AA}(2)$	0.371	0.0	0.050	0.341	0.0
μ_1	3.44	3.50	3.51	3.45	3.50
μ_2	3.21	3.22	3.25	3.21	3.23
σ	0.482	0.475	0.479	0.486	0.486
Difference in $\log_e$ likelihood	58.1	11.35	...	52.52	...

NOTE: Phenotype classification no. 3 is described in table 1.

TABLE 5

LOD SCORES FOR LINKAGE BETWEEN AFFECTIVE DISORDER AND 21 AUTOSOMAL MARKERS

Locus	No.	0.0	0.1	0.2	0.3	0.4
ABO	26	−22.14	−5.36	−2.01	−0.52	0.02
Rh	30	−14.01	−3.86	−1.72	−0.69	−0.22
MNS	30	−18.18	−0.35	1.39	1.26	0.55
K	11	−3.71	−0.60	−0.08	0.10	0.11
Fy	29	−5.98	−2.06	−0.97	−0.37	−0.10
Jk	10	−2.46	−0.82	−0.14	0.11	0.14
Le	6	−0.99	−0.55	−0.27	−0.11	−0.02
Se	22	−6.23	−1.13	−0.15	0.06	0.03
P	9	−4.49	−1.64	−0.68	−0.22	−0.02
Hp	25	−26.21	−3.21	−0.86	−0.08	0.04
ESD	12	−4.18	−1.72	−0.79	−0.33	−0.09
GPT	23	−9.55	−3.58	−1.44	−0.43	−0.04
Gc	16	−10.19	−3.82	−1.69	−0.66	−0.17
ADA	10	−3.70	−1.36	−0.72	−0.35	−0.12
AK	7	−5.14	−1.22	−0.34	−0.04	0.01
AMY_2	4	−3.51	−0.81	−0.34	−0.13	−0.03
PGM_1	19	−1.39	0.13	0.31	0.19	0.05
ACP	21	−12.87	−2.69	−1.22	−0.61	−0.26
GALT	10	−3.42	−1.33	−0.67	−0.32	−0.11
GLO	4	−1.32	−0.40	−0.20	−0.11	−0.05
PGD	4	0.36	0.29	0.20	0.10	0.03

NOTE: Affective disorder is phenotype model no. 3 as defined in table 1.

other phenotype models, except in the widest classification (no. 4 in table 1) where they are lower. A few isolated families have lod scores greater than 1.0 at other loci, but there is not significant heterogeneity of lod scores at these loci. Two families have lod scores of approximately 1.0 for the Hp locus, one family has a lod score of approximately 1.4 for the *ABO* locus, and another family has a score of 1.6 for the ACP locus. Whether or not these are chance results is uncertain. However, none of these results are strong enough to support the existence of a single locus for affective illness. In addition, our conclusions about the absence of linkage are limited by our lack of knowledge of the true mode of inheritance of affective disorder. As mentioned above, we did not find evidence in previous studies for linkage of major affective disorder to either X-chromosome marker loci [15, 16] or the *HLA* locus assuming either a dominant [18, 21] or recessive [21] mode of transmission of illness.

DISCUSSION

Our data show a relatively high prevalence of major affective illness in first-degree relatives of patients when compared with other studies. Despite this high prevalence, we cannot find any evidence from either segregation or linkage analysis that a single major locus accounts for the transmission of illness in these families.

In [4], we report that the prevalences of major affective disorders in first-degree relatives of probands are consistent with a multifactorial threshold hypothesis that allows for separate thresholds for UP, BP, and SA illness. It is difficult to compare the multifactorial model results with the present single-major-locus analyses since

the former analysis was based on prevalence data in first-degree relatives and therefore has little power in distinguishing multifactorial from single-major-locus hypotheses. Clearly, application of a mixed model to these pedigrees would be more powerful for resolving these genetic components.

It may be that the illness is heterogeneous and that some families are segregating for single genes. In our sample, we have several larger pedigrees in which over 60 individuals were examined. However, when segregation analyses were applied to these single pedigrees individually, there was no discrimination among hypotheses (i.e., all hypotheses had similar likelihoods and were not rejected [results not shown]). Thus, we have the dilemma that a large sample of families may be heterogeneous but that a single large family may not contain enough information to discriminate among hypotheses. This also seems to be true in the analysis of a single large UP pedigree by Crowe et al. [29]. In addition, because of assortative mating for affective disorders [47–49], even single large pedigrees may not be genetically homogeneous.

Another explanation for these results may lie in the limits of the power of the statistical methods. We showed by simulation [50] that even under a simpler model than the one used here the presence of a single major gene will not always be detected.

Other problems may be caused by cultural and environmental variables that affect the likelihood of a diagnosis being made. For instance, in our data we find that the prevalence of illness in older-generation, second-degree relatives is very low (approximately 6%). This is much lower than would be predicted under any genetic hypothesis and is thought to be related to cultural differences in the reporting of psychiatric symptomatology between the older generations and the current generations [3, 4]. However, even if we apply segregation analysis only to the first-degree relatives in the BP I sample, all single gene and environmental hypotheses are still rejected (phenotype models nos. 3 and 4, results not shown). Thus, while there may be a bias in pedigree data over generations due to cultural differences, it does not account for the failure to detect single-locus transmission of illness.

The question of the effect of ascertainment biases with respect to our results can be raised. In our study, second-degree and more distant relatives were examined only when illness appeared to be segregating. Nonetheless, *all* families were included in the analysis. This method of ascertainment did not strictly correspond to the sequential sampling method given by Cannings and Thompson [51] and shown to be unbiased. However, as stated above, all genetic hypotheses were rejected when only first-degree relatives were analyzed.

Recent linkage studies yielded contradictory results. Some investigators found evidence for linkage of BP and SA illness to the X-linked colorblindness and *G6PD* loci [9–14]. Data from our own sample were previously found to be inconsistent with linkage of BP illness to either the X-linked colorblindness locus [15] or to the *Xg* locus [16]. Recent data from a study of affective disorders in the Amish were not consistent with linkage to colorblindness [52]. Thus, X-chromosome transmission of BP affective disorder remains controversial. Turner and King published evidence for linkage of affective disorder to the *HLA* locus in a few

families [17], but their diagnostic methods may not be consistent with other studies. Our own sample did not support this linkage [18]. A study by Weitkamp et al. [19] found no evidence of linkage of UP illness in a single large pedigree to 29 marker loci, including HLA.

A more recent report from Weitkamp et al. [20] concluded that a major susceptibility gene for depression is located in the HLA region. However, this finding is true only for a subgroup of the sample. We criticized the analytic methods used in this study and have shown that additional data from our sample do not support any relationship between HLA and depression [21].

In view of our result and those from other recent segregation and linkage analyses of affective disorders [26, 27, 29], we can conclude that when using clinical criteria to define phenotypes a single two-allele locus does not account for the familial concentration of affective illness in the majority of families studied as a whole sample or in any of the subdivisions tested.

Whether or not there is heterogeneity in our data cannot be determined from these results. If there were a small proportion of families in our sample in which the illness was transmitted as a single major locus, this might not be detectable from segregation analysis. However, some investigators claim that there is genetic heterogeneity of affective disorders and that a *large* proportion of families represent a single-locus disorder. For example, Risch and Baron [53] argue that the BP illness-X-linkage data reported in the literature are heterogeneous and that the major affective illness in a large proportion of the BP and SA families is transmitted on the X chromosome. The limits of segregation analysis in the presence of a genetically heterogeneous population of families may be definable through simulation studies. The hypothesis of heterogeneity would be more convincing if supported by some clear clinical or biological differentiation of patients. We speculate that the nature of the transmission of major affective disorders in families will most likely be resolved by continuing to search for biological susceptibility traits and by considering some of the more complex modes of genetic transmission such as a mixed model or those involving two or more loci.

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