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Use of a Quantitative Trait to Map a Locus Associated with Severity of Positive Symptoms in Familial Schizophrenia to Chromosome 6p

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Summary

A number of recent linkage studies have suggested the presence of a schizophrenia susceptibility locus on chromosome 6p. We evaluated 28 genetic markers, spanning chromosome 6, for linkage to schizophrenia in 10 moderately large Canadian families of Celtic ancestry. Parametric analyses of these families under autosomal dominant and recessive models, using broad and narrow definitions of schizophrenia, produced no significant evidence for linkage. A sib-pair analysis using categorical disease definitions also failed to produce significant evidence for linkage. We then conducted a separate sib-pair analysis using scores on positive-symptom (psychotic), negative-symptom (deficit), and general psychopathology–symptom scales as quantitative traits. With the positive symptom–scale scores, the marker D6S1960 produced $P = 1.2 \times 10^{-5}$ under two-point and $P = 5.4 \times 10^{-6}$ under multipoint analyses. Using simulation studies, we determined that these nominal *P* values correspond to empirical *P* values of .034 and .0085, respectively. These results suggest that a schizophrenia susceptibility locus on chromosome 6p may be related to the severity of psychotic symptoms. Assessment of behavioral quantitative traits may provide increased power over categorical phenotype assignment for detection of linkage in complex psychiatric disorders.

Introduction

Schizophrenia is a serious neuropsychiatric illness affecting as much as 1% of the general population. Family, twin, and adoption studies have demonstrated that schizophrenia is predominantly genetically determined and has high heritability (Gottesman and Shields 1982; Kendler and Diehl 1993; McGuffin et al. 1994). Although no gene for schizophrenia has yet been identified, evidence suggestive of linkage to chromosome 6p (Straub et al. 1995; Wang et al. 1995), with supportive evidence from other groups (Moises et al. 1995; Schwab et al. 1995; Wang et al. 1996), has recently been achieved by use of both parametric and nonparametric methods. Together, these studies meet criteria for significant linkage (Lander and Kruglyak 1995). The positive results extend over a 40-cM region, with the strongest signals from two or more studies occurring at 6p23 (markers D6S274 and D6S285 [Moises et al. 1995; Schwab et al. 1995; Straub et al. 1995]) and with weaker results occurring at 6p21 (markers CAR and D6S291 [Moises et al. 1995; Schwab et al. 1995], closer to the HLA region. Other studies, using similar methods, produced negative or nonsignificant LOD scores (Antonarakis et al. 1995; Gurling et al. 1995; Mowry et al. 1995), possibly because of low statistical power.

The studies showing positive linkage to 6p used large samples of families from several geographic regions: 265 families from Ireland (Straub et al. 1995), 65 from Germany and Israel (Schwab et al. 1995), 5 from Iceland, and 65 from eight other countries (Moises et al. 1995). Diagnostic categories varied from narrow (schizophrenia and schizoaffective disorder [Moises et al. 1995; Schwab et al. 1995; Straub et al. 1995]) to very broad (Straub et al. 1995), including nonaffective psychotic disorders; schizotypal, paranoid, schizoid, and avoidant personality disorders; and affective psychotic disorders. Each of these disorders may be genetically related to schizophrenia (Kendler and Diehl 1993). Despite the use of a broad phenotype and large sample sizes, LOD scores in each of these studies were generally low, the largest being 3.51 (Straub et al. 1995), perhaps because of non-Mendelian inheritance, genetic heterogeneity, and/or genes that may be necessary but not sufficient to cause illness (Matthysse et al. 1992; Tsuang 1993; Farmer et al. 1994; Rutter 1994).

One way to increase power in linkage studies of complex disorders, regardless of the mode of inheritance, is

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to use phenotypes that include key dimensions of gene expression that may act as quantitative traits (Risch 1990; Matthysse et al. 1992; Faraone et al. 1995). Positive symptoms (disorganized thinking, hallucinations, and delusions) and negative symptoms (blunted affect, limited spontaneous speech, and social withdrawal) are fundamental components of schizophrenia (Andreasen et al. 1994). They are measurable in families and appear to be related to genetic predisposition to schizophrenia (Dworkin et al. 1991; McGuffin et al. 1991; Tsuang et al. 1991; Bassett et al. 1993), which suggests that they may be used as quantitative traits. In general patient samples, longitudinal studies indicate that these symptom clusters are stable during remission phases of schizophrenic illness over periods of 1–10 years (Malla et al. 1993; Rey et al. 1994; Arndt et al. 1995; Eaton et al. 1995). In previous studies, we have shown that these traits are measurable in familial schizophrenia in both affected and unaffected individuals, using a sensitive assessment instrument with good psychometric properties (Bassett et al. 1993). On the basis of the hypotheses that (1) different aspects of the schizophrenia phenotype may be primarily influenced by different genes and (2) nonschizophrenic carriers of a schizophrenia susceptibility allele may express symptom levels intermediate between those of individuals with schizophrenia and those of noncarriers, we have sought to apply a multidimensional, quantitative scale to phenotype assignment in our study of familial schizophrenia. We have therefore used both diagnostic classification and quantitative-trait measures to investigate linkage to markers on chromosome 6 in 10 Canadian families of Celtic descent.

Subjects and Methods

Families and Phenotype Assignments

Canadian families of Celtic decent were recruited for the linkage study if schizophrenic illness appeared to be segregating in a unilineal autosomal dominant manner (Bassett et al. 1993; Bassett and Honer 1994). Ten moderately large families ($n = 183$ subjects) were assessed and had DNA samples available for the current study. These subjects were all enrolled in this study after they had given informed consent, and all procedures conformed to human-subjects protocols approved by the University of Toronto and Rutgers University. Direct interviews using the Structured Clinical Interview for DSM-III-R (SCID) for major disorders, SCID-II for personality disorders, collateral information, and medical records were used to make consensus diagnoses on the basis of DSM-III-R criteria. Further details of the diagnostic and ascertainment methods have been described elsewhere (Bassett et al. 1993; Bassett and Honer 1994). The following diagnostic categories were used: narrow (schizophrenia and chronic schizoaffective disorder), broad (narrow plus nonaffective psychotic disorders and schizotypal personality disorder), and very broad, matching the Straub et al. (1995) D1–D8 classification (broad plus psychotic affective disorders and paranoid, avoidant, and schizoid personality disorder). There were 42, 65, and 70 affected individuals in the narrow, broad, and very broad diagnostic categories, respectively.

A previous investigation had indicated that measures of positive and negative symptoms may be more sensitive to genetic risk than is diagnostic classification (Bassett et al. 1993). The Positive and Negative Syndrome Scale (PANSS) (Kay 1987) is a 30-item scale with sound psychometric properties designed to assess core signs and symptoms of schizophrenia, on a severity continuum from absent to extreme; the PANSS provides measures of 7 positive symptom–scale items related to psychosis and thought disorder (e.g., suspiciousness and disorganized thinking), 7 negative symptom–scale items related to deficits in affect and behavior (e.g., blunted affect and social withdrawal), and 16 general psychopathology (GP) items related to general severity of illness (e.g., anxiety and somatic concerns). PANSS ratings were performed by a psychiatrist when subjects were in a stable, nonacute illness state, usually at the time of diagnostic interview. Individual items were summed to give total scores for the positive-symptom, negative-symptom, and GP scales. PANSS data were available on 166 subjects. Five of these subjects were excluded because the cross-sectional PANSS assessments were confounded by the presence of other, irreversible neuropsychiatric disorders such as stroke and dementia, resulting in a sample of 161 subjects with usable PANSS scores. Figure 1 illustrates the distribution of the scores of this sample, for affected and unaffected subjects, using the narrow diagnostic classification.

Genotyping

DNA was extracted from blood samples or lymphoblastoid cell lines by use of the GenePure system (Gentra Systems). DNA from each subject was genotyped by use of the 23 chromosome 6 markers of the Weber V6 screening set (Research Genetics), as well as by use of 5 additional chromosome 6p markers (D6S296, D6S277, D6S470, D6S259, and D6S285) used in previous linkage reports on schizophrenia (Straub et al. 1995). PCR amplifications were performed in a reaction volume of 12 μl containing 40 ng of template DNA, 0.2 mM each of dATP, dGTP, and dTTP, 1.25 μM dCTP, 25 nM ^{32}P-α-dCTP, 12 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, and 0.12 units of *Taq* DNA polymerase (Promega). Thermocycling was conducted by use of a MJ Research PTC-100 thermocycler, with an initial denaturation step of 5 min at 93°C; followed by 30 cycles of 1 min at 93°C, 1 min at 55°C, and 1 min at 72°C; followed by

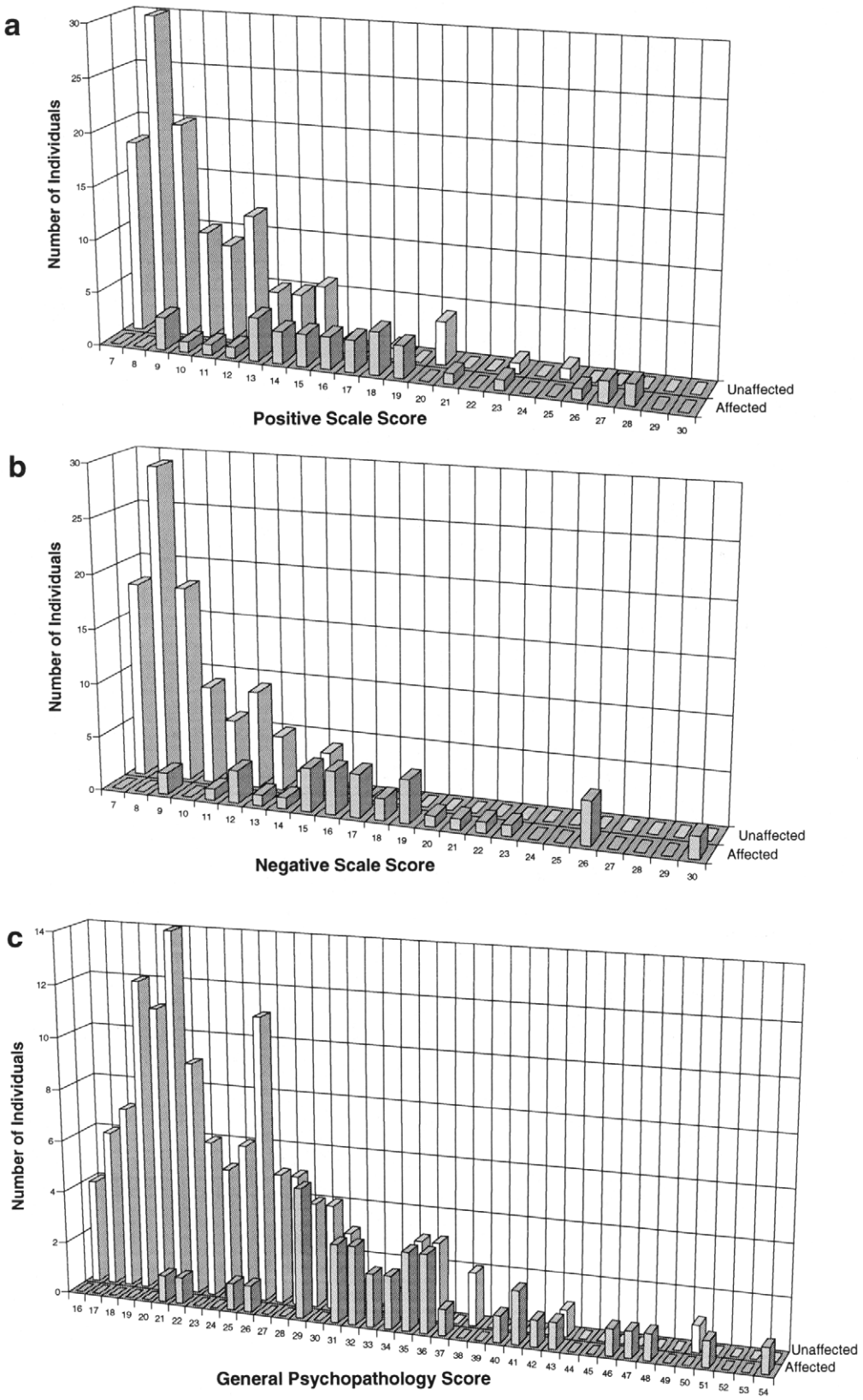

Figure 1 Distribution of PANSS total scale scores for affected and unaffected subjects, under the narrow diagnostic classification of schizophrenia. The numbers of affected subjects (*gray-shaded bars*) and unaffected subjects (*unshaded bars*), with values for each of the PANSS scales, are presented for the (*a*) positive symptom–scale, (*b*) negative symptom–scale, and (*c*) GP symptom–scale scores of PANSS, which have minimum possible values of 7, 7, and 16, respectively.

a final extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis on 6% nondenaturing acrylamide gels and by subsequent autoradiography, at room temperature, with Dupont Reflections film. All gels included reference DNA samples (CEPH individuals 1331-01 and 1331-02) to allow comparative sizing. To minimize the chance of errors due to sample mishandling, all subject DNA samples were maintained in racks of tubes compatible with standard 8×12 microtiter plates. All subsequent stages of sample handling, from PCR analysis through loading of the acrylamide gels, were done by use of multichannel pipettes and syringes.

Scoring was performed by visual inspection by two independent raters blind to subject phenotype. In the case of discrepant allele interpretation, the autoradiographs were reread by the two readers, and, if a discrepancy remained, PCR and electrophoresis of the samples in question were repeated. Certain genotypes, although being concordantly scored, nonetheless were not consistent with Mendelian inheritance. For these samples, the genotyping procedure was repeated, from the PCR stage. In all cases in which the apparent non-Mendelian inheritance persisted, it could be explained by a one-repeat-unit change in size of the microsatellite marker. Since the source of the original allele could not always be determined, we consistently excluded from our analysis any genotypes that exhibited evidence of size instability.

Statistical Analyses

Parametric analyses were conducting by use of LINKAGE version 5.2 programs. Five models were analyzed, two each for the narrow and broad diagnostic classifications and one for the very broad classification. Under the narrow diagnostic classification, the dominant model was schizophrenia susceptibility–allele frequency (P_A) .009 and disease penetrance (f) .75, .50, and .001 for disease homozygotes (AA), heterozygotes (Aa), and normal homozygotes (aa), respectively; the recessive model was $P_A = .13$, $f(AA) = .50$, $f(Aa) = .0015$, and $f(aa) = .0015$. Under the broad diagnostic classification, the dominant model was $P_A = .014$, $f(AA) = .90$, $f(Aa) = .80$, and $f(aa) = .009$, and the recessive model was $P_A = .20$, $f(AA) = .60$, $f(Aa) = .01$, and $f(aa) = .01$. The very broad diagnostic classification was analyzed under the intermediate "Pen" model of Straub et al. (1995), with $P_A = .035$, $f(AA) = .85$, $f(Aa) = .425$, and $f(aa) = .0108$. Two-point linkage analyses were conducted by use of MLINK, at recombination fraction (θ) values of 0, .01, .05, .1, .2, .3, and .4. Heterogeneity testing was conducted on these results by use of the HOMOG program. Allele frequencies for the analysis were estimated by use of a set of 25 unrelated subjects from these families. Since the distribution of PANSS scores in individuals with presumably normal genotypes is unknown, we were unable to construct a model to examine PANSS scores as a quantitative trait by using a parametric analysis.

Sib-pair analyses were conducted by use of the Genetics Analysis System (GAS), version 2.0 (Young 1995). The SIBHE and SIBIHE modules of GAS were used for two-point and multipoint analyses, respectively. These modules implement the Elston-Haseman algorithm for an identity-by-descent analysis of quantitative traits (Haseman and Elston 1972), which makes no assumptions about the underlying distribution of the quantitative traits. This algorithm compares the square of the difference in the value of the quantitative trait for all sibling pairs, ranging from highly concordant to highly discordant. Total scores on the positive-symptom, negative-symptom, and GP-symptom scales of the PANSS were each used as quantitative traits for sib-pair analysis with the 161 subjects on whom usable PANSS data were available. The Elston-Haseman algorithm may also be applied to the analysis of categorical data, by setting the squared trait difference equal to 0 for concordant sib pairs and equal to 1 for discordant sib pairs (Tran et al. 1995). In GAS, this is easily accomplished by coding unaffected individuals with the value 0 and affected individuals with the value 1 and then running the standard SIBHE and SIBIHE routines. Sib-pair analyses were thus conducted under the categorical diagnostic classifications of narrow, broad, and very broad, described above. Analyses were first conducted by use of categorical diagnostic data for all 183 subjects and then, for better comparison with the quantitative-trait analysis, were repeated for the 22 individuals with unavailable or unusable PANSS data coded as phenotype unknown.

The SIBIHE module was used for sib-pair interval mapping, with information from adjacent markers being used to infer missing or ambiguous allele sharing. This routine calculates the sharing probabilities in four steps. First, the loci at which the sharing status is definitely known are stored. Next, the estimated sharing at ambiguous intercrosses is calculated. Unknown-sharing-status loci are then interpolated from the results of the first two steps, and, finally, interloci values are interpolated on the basis of the results of the first three steps. Further details of these algorithms may be found in the GAS 2.0 analysis modules manual (Young 1995).

For the Weber-screening-set markers, multipoint analyses were conducted by use of θ values from the CHLC's Weber V6 Screening Set Extended Map's Recombination-Minimization Map Data (table 2), obtained from the CHLC website (http://www.chlc.org). Since no single map was available that integrated these markers with the additional markers from chromosome 6p that were genotyped, data from the additional markers were examined in a separate multipoint analysis using the fol-

Table 1

Z_{max} for Two-Point Linkage Analysis of Schizophrenia, with Chromosome 6 Markers

Genetic Model and Diagnostic Classification	Marker Locus	Z_{max}	θ_{max}
Dominant:			
Narrow	D6S477	.31	.3
Broad	D6S1040	.14	.3
Recessive:			
Narrow	D6S1040	.77	.1
Broad	D6S1003	1.19	.2
Pen:			
Very broad	D6S1040	.24	.2

lowing genetic map, presented by Straub et al. (1995): D6S477–.061–D6S296–.007–D6S277–.019–D6S470–.074–D6S259–.059–D6S285.

For all sib-pair analyses, the DFWEIGHT option was used to compensate for multipair sibships. In the assessment of the significance of the least-squares best-fit line, N pairs in a sibship contribute $(N - 1)$ df—instead of N df, which is used when all data points are independent. N and df were as follows: for two-point analyses, 15–164 pairs and df 6–49, depending on the informativeness of markers; for quantitative-trait multipoint analyses using the PANSS scores, 324 pairs and df 84 for the Weber V6 markers and 197 pairs and df 59 for the additional 6p markers; and, for the categorical multipoint analyses using all subjects, 379 pairs and df 98 for the Weber V6 markers and 235 pairs and df 70 for the additional 6p markers.

To assess the empirical P value associated with the nominal P values generated by the nonparametric analyses, simulation studies were conducted by use of SIMULATE (Terwilliger and Ott 1994), a software program that will simulate genotypes in family members for a map of linked markers that are unlinked to a given affection-status locus. Since the program does not use affection status–locus information for the generation of marker data, it is easily modified to accept quantitative-trait values instead. The simulation studies were conducted to allow for the multiple testing involved in the analysis of the multiple marker loci and the three quantitative and three categorical traits. Ten thousand unlinked replicates of pedigree sets with the subjects' original PANSS and categorical affection-status data were simulated, and each replicate was analyzed by use of the same procedures as were used for the actual linkage data. Each replicate was separately analyzed by use of the quantitative and categorical phenotypes, with the most significant P values from each set of two-point and multipoint analyses extracted and compiled into a single distribution. This produced a total of four distributions: one for the two-point analyses and one for the multipoint analyses, for each of the categorical traits and the quantitative traits. The smallest nominal P value from the real analysis was then compared with these distributions of 10,000 simulated P values, to determine how often such a P value would be expected by chance in an unlinked data set. This is reported as the empirical P value. The smallest P value from the entire set of corresponding categorical-trait and quantitative-trait analyses was also extracted and assembled into a single distribution, to allow for the determination of the empirical significance of the single smallest nominal P value across the entire set of six phenotypes.

Results

Under the diagnostic schemes tested, the parametric analyses produced no significant evidence of linkage to chromosome 6 in these families. Under the dominant, the recessive, and the intermediate Pen model of Straub et al. (1995), two-point analysis of 28 markers spanning chromosome 6 produced no significant evidence for linkage under the hypothesis of homogeneity (table 1). Analysis of these results by the HOMOG program produced no significant evidence for heterogeneity: no odds ratio favoring linkage under the hypothesis of heterogeneity was >2.1.

Sib-pair analysis conducted with use of the narrow, broad, and very broad diagnostic categories produced no significant evidence for linkage, with either the two-point approach or the multipoint approach. Results of the multipoint sib-pair analyses using the Weber-screening-set chromosome 6 markers are presented in table 2. Separate multipoint analyses with the 6p markers D6S477, D6S296, D6S277, D6S470, D6S259, and D6S285, used in other schizophrenia linkage studies, are presented in table 3. The smallest nominal P values were .0096 for the two-point analysis and .038 for the multipoint analysis, in both cases with marker D6S1009 and the very broad diagnostic classification. These correspond to empirical P values of .35 and .42, respectively. To allow better comparability with the analyses using the PANSS ratings, these analyses were also conducted with any subjects lacking PANSS data coded as categorical phenotype unknown. The removal of the phenotype data on these 22 subjects did not significantly alter any results.

In contrast, both the two-point and multipoint sib-pair analyses using the PANSS scale scores as quantitative traits produced significant evidence (empirical $P < .05$) for linkage. The results for the two-point and multipoint analyses were similar. The two-point analysis produced significant evidence (nominal $P = 1.2 \times 10^{-5}$, empirical $P = .034$) for linkage to marker locus D6S1960, for the positive symptom–scale score only. The multipoint analysis produced significant evidence

Table 2

Results of Multipoint Sib-Pair Analysis Using Categorical Diagnostic Definitions and PANSS Scale Scores and Chromosome 6 Markers (Weber V6 Screening Set)

		Nominal *P*					
		Categorical Traits			Quantitative Traits		
Intermarker θ[a]	Locus[b]	Narrow	Broad	Very Broad	Positive Symptoms	Negative Symptoms	GP Symptoms
.04	D6S477	.049	.54	.48	.52	.0060	.11
.11	D6S263	.34	.58	.64	.42	.048	.052
.17	D6S1006	.64	.58	.50	.31	.017	.020
.06	D6S1050	.55	.52	.42	.092	.028	.020
.10	D6S1281	.65	.63	.50	.13	.070	.047
.06	D6S1019	.66	.57	.39	.062	.16	.23
.14	D6S1017	.84	.77	.60	.062	.25	.13
.04	D6S1280	.91	.77	.79	.000030	.17	.0088
.04	D6S1960	.83	.59	.60	.0000054 [c]	.071	.00032
.06	D6S1053	.78	.61	.59	.000070	.061	.0037
.06	D6S1031	.90	.78	.74	.00030	.14	.0070
.11	D6S1270	.93	.82	.74	.00062	.15	.010
.10	D6S1056	.93	.84	.69	.017	.17	.036
.06	D6S1021	.91	.71	.57	.059	.076	.038
.10	D6S474	.93	.54	.39	.11	.082	.049
.01	D6S1040	.91	.21	.11	.21	.18	.018
.08	D6S1009	.93	.099	.038	.39	.14	.12
.14	D6S1003	.97	.29	.23	.53	.34	.25
.07	D6S1007	.96	.77	.75	.47	.74	.29
.07	D6S305	.99	.76	.88	.49	.79	.32
.12	D6S1277	.97	.83	.89	.63	.81	.51
.03	D6S503	.98	.99	.99	.57	.75	.39
	D6S1027	.98	.98	.99	.56	.78	.41

[a] Between each contiguous pair of loci shown.
[b] Arranged from most telomeric on 6p (D6S477) to most telomeric on 6q (D6S1027).
[c] Significant at empirical $P = .0085$.

for linkage (nominal $P = 5.4 \times 10^{-6}$, empirical $P = .0085$) to D6S1960, again only for the positive symptom–scale score. When all sib-pair analyses, quantitative and categorical, are considered together, this multipoint linkage result corresponds to a *P* value of .011. Results of the multipoint sib-pair analyses using the Weber-screening-set chromosome 6 markers are presented in table 2; and the results of the multipoint analyses with the additional chromosome 6p markers are presented in table 3. Although none of the empirical *P* values from the analyses of the 6p markers were significant, most of the nominal *P* values were <.05.

Discussion

To date, individual linkage studies of schizophrenia have produced mixed results, characterized by suggestive, but not significant, results and frequent lack of rep-

Table 3

Results of Multipoint Sib-Pair Analysis Using Categorical Diagnostic Definitions and PANSS Scale Scores and Chromosome 6p Markers Used in Previous Studies

		Nominal *P*					
		Categorical Traits			Quantitative Traits		
Intermarker θ[a]	Locus[b]	Narrow	Broad	Very Broad	Positive Symptoms	Negative Symptoms	GP Symptoms
.061	D6S477	.10	.69	.62	.13	.0018	.036
.007	D6S296	.72	.83	.86	.021	.029	.0024
.019	D6S277	.50	.67	.72	.018	.033	.0021
.074	D6S470	.71	.83	.87	.012	.045	.0017
.059	D6S259	.83	.86	.75	.0080	.077	.0056
	D6S285	.82	.84	.78	.026	.14	.030

[a] Between each contiguous pair of loci shown.
[b] Arranged from most telomeric (D6S477) to most telomeric (D6S285) on 6p.

lication across studies. The finding of potential linkage to chromosome 6p has been the most reproducible of these findings, yielding suggestive or marginally significant results in several large samples (Moises et al. 1995; Schwab et al. 1995; Straub et al. 1995). Although different diagnostic and analytic approaches have been used, all of these studies share the basic methodology of assigning subjects to clinically derived categorical definitions of illness, which may not be as powerful as linkage analyses using quantitative traits. Our results suggest that using selected quantitative behavioral traits may provide significantly increased power in linkage studies of schizophrenia.

Our initial parametric and nonparametric analyses using categorical diagnoses failed to produce any significant evidence of linkage to chromosome 6, despite using a sample of ethnic origin similar to that studied by Straub et al. (1995). To make our study as comparable as possible to the findings of Straub et al. (1995), we included a parametric analysis using the same diagnostic classification and inheritance model as was used for the maximum LOD score (Z_{max}) obtained in their study. Although we failed to find significant evidence for linkage with this genetic model, it must be noted that our sample is only 12% of the size of the sample studied by Straub et al. (1995). Given that their Z_{max} was 3.51, with an estimated 60% of families unlinked, it would not be surprising for our sample of 10 families to be too small to either detect linkage or statistically support the hypothesis of heterogeneity. Additionally, although our sample has many sibling pairs, because of the distribution of affected individuals, there are relatively few pairs of affected siblings, with a maximum of 75 affected sib pairs, under the very broad diagnostic classification. Because of this, we chose to employ the Elston-Haseman algorithm to analyze our categorical sib-pair data, thus utilizing affected, unaffected, and discordant pairs. However, it should be noted that this analysis is not directly comparable to the chromosome 6 affected-sib-pair analyses reported by other groups.

The use of positive- and negative-symptom scores as quantitative traits introduces into the analysis two changes that may increase the power to detect linkage. First, the use of a quantitative instead of categorical trait is associated with increased power in linkage analysis. Second, the assessment of symptom clusters captures phenotypic elements different than those described by categorical diagnoses. The specificity of the significant linkage results to the positive-symptom scores but not to the negative-symptom scores suggests that the power increase found in our study may represent more than just the generally increased power of quantitative-trait analyses. Positive- and negative- symptom clusters appear to be partially overlapping dimensions of a schizophrenia phenotype in families and may have different susceptibility loci (Bassett et al. 1993). With regard to schizophrenia, positive symptoms may be more strongly correlated with an underlying genetic vulnerability located on chromosome 6p than are negative symptoms. Alternatively, negative symptoms related to a schizophrenia locus may be more susceptible to noise from unrelated disorders such as alcoholism in schizophrenia families (Bassett et al. 1993), with linkage thereby obscured. Further studies are needed to resolve these issues.

Another important contrast between the current study and previously published nonparametric analyses of schizophrenia is the use of simulation studies to evaluate the nominal P values generated by the analysis programs. Many sib-pair analyses of schizophrenia have previously interpreted nominal P values of $<.05$ as being significant. Lander and Krugylak (1995) recently suggested guidelines for P values that should be taken as significant for genomewide linkage studies in sib-pair analysis, with $P = 2.2 \times 10^{-5}$ offered as the threshold for a significant finding and with $P = 7.4 \times 10^{-4}$ as the threshold for suggestive findings. Although these guidelines are certainly helpful, they do not take into account the effects of all the variables that may be acting in a particular study. In the current study we determined empirical P values from the distribution of nominal P values generated by the analysis of a series of unlinked, simulated data sets. In doing so, we have corrected for the multiple testing associated with both genotyping a dense genetic map and using multiple diagnostic models. This method thereby produces a better estimate of the chances that the finding represents a false-positive linkage.

The markers demonstrating significant linkage to positive-symptom scores in this study are centered around the 6p11-p21 region, slightly more centromeric to the 6p24-21 region previously implicated in schizophrenia (Moises et al. 1995; Schwab et al. 1995; Straub et al. 1995; Wang et al. 1995). Several explanations for these slightly different localizations are possible. First, two distinct loci contributing to schizophrenia vulnerability could be located on chromosome 6. Second, the differences in phenotypic classification may be producing imprecision in susceptibility-locus localization. Finally, most of the markers used in this and previous studies were different, with many of the cited studies not reporting results on markers extending into the p11 region of chromosome 6, the area of our most significant linkage finding. Also of note is that, although none of the more distal 6p markers used provided significant evidence for linkage when empirical P values were considered, many of the nominal P values for linkage of the PANSS scale scores to these markers were $<.05$ and therefore would likely have been reported as significant according to methodology used in prior studies. This result further underlines the value of using simulation

or other statistical methods to determine the true significance associated with linkage-analysis results.

Schizophrenia is a complex disorder, with unclear inheritance and evidence for multiple components to the clinical phenotype. Previous linkage studies of markers from chromosome 6p, using diagnostic categories as phenotypes, have resulted in suggestive or marginally significant findings in samples of 358–1,408 individuals (Moises et al. 1995; Schwab et al. 1995; Straub et al. 1995). Although the validity of a quantitative-trait approach to schizophrenia has not yet been proved, we have been able to identify a significant linkage finding with a sample of only 183 subjects, by performing a quantitative-trait analysis using certain component features of the schizophrenia phenotype. The use of the three scales of the PANSS represents a relatively straightforward approach to examination of different aspects of schizophrenia. Additional refinement of the components of the schizophrenia phenotype will allow for further increases in power and accuracy in linkage studies of this challenging disorder.

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