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Suggestive Evidence for a Schizophrenia Susceptibility Locus on Chromosome 6q and a Confirmation in an Independent Series of Pedigrees

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We have investigated whether there is a locus on chromosome 6 that confers an increased susceptibility to schizophrenia using a two-stage approach and nonparametric linkage analysis. Allele sharing identical by descent (IBD) and multipoint maximum likelihood score (MLS) statistics were employed. Results from two tested data sets, a first data set, or genome scanning data set, and a second replication data set, show excess allele sharing for multiple markers in 6q, a chromosomal region not previously reported as linked to schizophrenia. In our genome scanning data set, excess allele sharing was found for markers on 6q13–q26. The greatest allele sharing was at interval 6q21–q22.3 at marker D6S416 (IBD percentage 69; $P = 0.00024$). The multipoint MLS values were greater than 2.4 in the 11.4-cM interval delimited by D6S301 and D6S303, with a maximum value of 3.06 close to D6S278 and of 3.05 at D6S454/D6S423. We did not confirm, however, the previously described linkage in 6p, when tested in the systematic genome scanning data set. The replication data set also showed excess allele sharing in chromosomal area 6q13–q26, which overlapped with the aforementioned positive linkage area of the genome scanning data set. The highest sharing of the second data set was at D6S424 (IBD percentage 64; $P = 0.0004$), D6S283 (IBD percentage 62; $P = 0.0009$), and D6S423 (IBD percentage 63; $P = 0.0009$). Multipoint MLS analysis yielded MLS values greater than 1 in an area of about 35 cM, which overlaps with the MLS multipoint area of linkage from the genome scanning data set. The multipoint MLS at the D6S454/D6S423 locus was 2.05. In the second data set, the maximum multipoint MLS was located about 10 cM centromeric from the maximum of the genome scanning data set, at the interval D6S424–D6S275 (2.35). Our results provide very suggestive evidence for a susceptibility locus for schizophrenia in chromosome 6q from two independent data sets.

INTRODUCTION

Schizophrenia affects approximately 1% of the population (McGue *et al.*, 1983) with a severe chronic disorder characterized by social withdrawal, illogical thinking, delusions, and hallucinations and is accompanied in various degrees by social, emotional, and behavioral disruption. Family, twin, and adoption studies have shown that genetic factors markedly increase the risk for schizophrenia (Bertelsen, 1985; Gershon *et al.*, 1988; Gottesman and Bertelsen, 1989; Kendler, 1988). The risk of the disease in siblings of affected individuals is about 10 times higher than the population prevalence (Kendler *et al.*, 1993; Maier *et al.*, 1993). The transmission of schizophrenia is unlikely to be caused by a single gene with very large effect (O'Rourke *et al.*, 1982). Polygenic or oligogenic multifactorial inheritance (Tsuang *et al.*, 1991) probably accounts for most of the genetic susceptibility (Risch, 1990).

We have employed a two-stage approach. First, as part of our genome-wide search for schizophrenia susceptibility genes, we have assembled a sample of families for systematic genome scanning, consisting of 53 families with one or more affected sib pairs (ASPs) with schizophrenia or schizoaffective disorder [*Diagnostic and Statistical Manual of Mental Disorders*, third edition, revised (DSM-III R)] (APA, 1987) for a total of 81 ASPs (Table 1). We have mapped these families with 41 markers spanning chromosome 6. We employed nonparametric linkage analyses because of the uncertainty about the mode of inheritance of schizophrenia. Second, we investigated a chromosomal area with nominal P values less than 0.01 (two-point nonparametric linkage test) with an additional data set (replication data set) consisting of a subset of 69 families including 109 ASPs

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TABLE 1

Family Categories of First Data Set

Affected offspring	Families with both parents	Families with a parent missing	Families (n)	Total ASPs $[n(n-1)]/2$
2	30	18	48	48
3	2		2	6
4	2		2	12
6	1		1	15
Total families	35	18	53	
Total ASPs $[n(n-1)]/2$	63	18		81

obtained from the National Institute of Mental Health (NIMH) Schizophrenia Genetics Initiative (Table 2). Our results provided supportive evidence for linkage of schizophrenia to 6q in two data sets, but did not confirm a linkage to 6p (Straub *et al.*, 1995) in our systematic scanning sample.

MATERIALS AND METHODS

Genome Scanning Data Set (First Data Set)

A total of 53 families were studied. Minimal criteria for inclusion in the study were two participating siblings affected with either schizophrenia or schizoaffective disorder with chronic psychosis. Families were recruited mainly by advertisements through an advocacy group (National Alliance for the Mentally Ill) and by clinical collaborators. This study was approved by the NIMH Institutional Review Board, and all participants gave written informed consent. Diagnostic resources included a semistructured diagnostic interview [Schedule for Affective Disorders and Schizophrenia–Lifetime Version (SADS-L)] (Endicott and Spitzer, 1978), systematic review of medical records (at least one medical record per affected individual; average number of medical records 2.8), and family history interviews (Gershon *et al.*, 1988). Final DSM-III R diagnosis (APA 1987) based on all sources of information was performed by the best estimate diagnosis method. All families were collected in the United States. Thirty-three families were previously described (DeLisi *et al.*, 1987; Gershon *et al.*, 1988). An additional 20 families were collected by the same methods. The 53 families contained 81 ASPs considering all possible pairs (Table 1). In this clinical sample the age of onset of schizophrenia, counted as first hospital admission for symptoms of the disease, was 21.6 ± 8.1 years. The ethnic composition was (defined as predominant ethnic composition per family): Caucasian 77.1%; Slavic 7.0%; African-American 3.5%; and other 12.4%. Of all the possible ASP combinations, the ASP types were schizophrenia–schizophrenia, 43.7%; schizophrenia–schizoaffective, bipolar type 31.0%; schizophrenia–schizoaffective, depressive type 10.3%; schizoaffective, depressive type–schizoaffective depressive type 4.6%; schizoaffective, bipolar type–schizoaffective, depressive type 3.4%; schizoaffective, bipolar type–schizoaffective, bipolar type 6.9%.

Replication Data Set (Second Data Set)

Sixty-nine families with an affected sib pair with schizophrenia or schizoaffective disorder were obtained from the NIMH Schizophrenia Genetics Initiative collection. These pedigrees were ascertained at three institutions—Columbia University, Harvard University, and Washington University (St. Louis). Data collection included a structured interview with the Diagnostic Interview for Genetic Studies (Nurnberger *et al.*, 1994); family history data were collected by using the Family Instrument for Genetic Studies; and diagnosis was made by DSM-III R. A pedigree was enrolled if it contained at least one pair such that one member was diagnosed with schizophrenia and the other was diagnosed with schizophrenia or schizoaffective, depressive-type disorder. Families including siblings with schizoaffective, bipolar-type diagnoses were excluded from this collection. The age of onset of schizophrenia (n = 142, no available information on 35 affecteds) counted as first hospital admission for symptoms of the disease was 23 ± 6.6 years and counted as age of first psychosis was 20.5 ± 7.3. The ethnic composition of the subset of families used by us for chromosome 6 linkage mapping is 51.0% Caucasian, 35.0% African-American, and 14.0% other ethnic origins. Of all the possible ASP combinations, the ASPs types were schizophrenia–schizophrenia 73.0%; schizophrenia–schizoaffective, depressive type 25.0%; and schizoaffective, depressive type–schizoaffective, depressive type 2.0%.

Chromosomal Markers

We studied 41 microsatellites markers with average heterozygosity of 0.73 ± 0.11 across a region of about 233 cM on chromosome 6. Microsatellite markers were primarily selected from the Généthon map (Dib *et al.*, 1996). Results from four chromosome 6p24–p22 markers (D6S296, D6S470, D6S259, and D6S285) in a subset of 44 of our families were previously reported (Schizophrenia Collaborative Linkage Group for Chromosomes 3, 6, and 8, 1997). Closely spaced markers were used for 6p24–p22 and in a region of chromosome 6q that showed a P value $\leqslant 0.01$ in two-point ASP analysis with one of our screening markers (D6S261).

Laboratory Techniques

All affected sibling pairs with DNA and available biological parents were genotyped. The genotyping of the genome scanning data set was performed by radioactive methods as previously described (Gejman *et al.*, 1993). Typically, two sets of primers were included in each reaction. Polymerase chain reactions (PCRs) were prepared by a robot (Biomek 2000, Beckman Instruments). For the study of the replication data set, we have employed fluorescence-based genotyping. Genotyping was performed using an ABI Prism 377 DNA sequencer (Perkin–Elmer). DNA amplification conditions of fluorescently labeled primer panels were as recommended by the manufacturers. After PCR, aliquots of PCR products were pooled into specific groups with a Hydra 96 microdispenser (Robbins Scientific). Electrophoretic data were collected and analyzed using Genescan and Genotyper software (ABI) and automated binning software (Ghosh *et al.*, 1997). For both methods, each genotype was read by two independent readers, blind to diagnosis, and inconsistencies were resolved in the presence of a senior investigator (P.V.G.).

Genetic Analyses

Allele frequencies for all markers were estimated from the families genotyped for this study using the program ILINK (Cottingham *et al.*, 1993; Lathrop and Lalouel, 1984; Schaffer *et al.*, 1994). Genetic analyses and checks for Mendelian inheritance provided information for double or multiple recombinants. Distance between markers was estimated from the Généthon map and by two-point linkage analysis; the order of markers was compatible with those of the Généthon map (Dib *et al.*, 1996).

Sibpair Analysis

Allele sharing method. This method scores marker alleles transmitted from each informative parent to a pair of affected sibs as shared or unshared. The proportion of allele sharing is estimated by N_1/m, where $m = N_1 + N_0$. N_1 and N_0 are the total number of shared and unshared alleles IBD, respectively, and m is the total number of informative meioses (number of heterozygous parents with IBD status unambiguously determined in the ASPs). The observed distribution of shared to unshared alleles IBD is compared to the expected distribution (i.e., $N_1 = N_0 = m/2$), under the null hypothesis of no linkage between marker and disease, using a one-sided χ^2 test,

TABLE 2

Family Categories of Second Data Set

Affected offspring	Families with both parents	Families with a parent missing	Families (n)	Total ASPs $[n(n-1)]/2$
2	17	37	54	54
3	1	12	13	39
4		1	1	6
5		1	1	10
Total families	18	51	69	
Total ASPs $[n(n-1)]/2$	20	89		109

$\chi^2 = (N_1 - N_0)^2/m$, with 1 degree of freedom. This method is implemented in the SIBPAIR program of the Analyze package (Terwilliger and Ott, 1993).

Maximum likelihood score (MLS). The MLS method (Risch, 1990b) computes a test statistic T, which is analogous to a lod score test (i.e., the $\log_{10}$ of T is the $\log_{10}$ of the odds in favor of linkage). The statistic T is the ratio of the likelihood of the observed marker information among ASPs maximized as a function of the three IBD (0, 1, or 2) probabilities (Z) to the likelihood of the marker data under the null hypothesis of no linkage (i.e., IBD probabilities set equal to $\frac{1}{4}$, $\frac{1}{2}$,and $\frac{1}{4}$). Holmans (1993) has shown that the power of the test is increased when imposing constraints among the Z parameters such as the possible triangle test ($2Z_0 \leqslant Z_1$ and $Z_1 \leqslant 0.5$) and that the resulting statistic distribution is a mixture of χ^2 with 1 and 2 degrees of freedom. The MLS tests were performed using the computer program MAPMAKER/SIBS version 2.1 (Kruglyak and Lander, 1995).

In our primary analyses we have considered all the possible ASPs as independent. However, the significance levels of these results can be biased at the extremes of the distribution. We corrected for this in two ways. First, we have employed a weighting function that weights by a factor = $2/r$ (r is the total number of affected siblings within a sibship) the contribution of each affected pair of siblings (Hodge, 1984). For this analysis, although the total number of ASPs is used, the statistic is the sum of the weighted contributions. Second, for the most significant markers we have computed the empirical P values that give a good approximation to the true significance of the results, although they involve a substantial amount of simulation. We thus estimated the empirical P values associated with our two-point ASP linkage results by simulating under the null hypothesis of no linkage marker data among ASPs in 5000 replicates only for the most significant markers.

RESULTS

The characteristics of the families employed in the two data sets are given in Tables 1 and 2. In the first stage, we screened all of chromosome 6 using 53 families with a total of 81 ASPs in the genome scanning data set of families, and we analyzed the results with two-point and multipoint nonparametric linkage analyses. The genotyping included 17 markers on 6p and 24 markers on 6q.

To minimize the total number of statistical tests, we used a single affection status model that included individuals with schizophrenia or schizoaffective disorder as affected. The results of the two-point analyses for the genome scanning data set are given in Table 3. Within the 6p area, no locus had a $P < 0.05$. On the other hand, a cluster of markers over a broad region of 6q (~60 cM) showed P values < 0.05. The strongest evidence for linkage was observed at D6S416 ($P = 0.00024$) and at D6S474 ($P = 0.0006$). The empirical P values in 5000 replicates were only slightly larger than the theoretical P values (D6S416 empirical $P = 0.0005$; D6S474 empirical $P = 0.0045$), indicating that our nominal P value is robust to deviations from independence assumptions. When a weighting function was applied to the two-point analyses (Hodge, 1984), the significance levels were reduced but the results remained positive at $P < 0.01$ (all families included: D6S416, $P = 0.0097$; and D6S474, $P = 0.0035$).

Allele frequencies do not affect the IBD sharing when both parents are genotyped but may influence sharing when families lack genotypes in one or both parents. Underestimated allele frequencies can lead to bias in favor of sharing and produce false-positive results or to a decreased power of detection and produce false-negative results (Holmans and Clayton, 1995; Tores *et al.*, 1996). To determine the potential for these artifacts in our sample, we have performed analyses with the subset of families with both parents available for genotyping (data not shown). Interestingly, these analyses showed an increased sharing and significance for most of the markers of the 6q13–q26 region. The most significant markers (unweighted) were D6S416 (IBD percentage = 69, $P = 0.00019$) and D6S474 (IBD percentage = 64, $P = 0.00058$). Two-point MLS results were consistent with the results obtained with the χ^2 sharing method (data not shown). The highest MLS values were obtained at D6S416 (MLS 3.11) and D6S474 (MLS 3.6), both results highly suggestive of linkage. The MLS values, as expected, are reduced when a weighting function is employed (D6S474, MLS 2.6; D6S416, MLS 1.44).

To use information from all the markers from a chromosomal area together, we performed multipoint ASP analyses using the MLS method. Multipoint analysis increases the power of detecting linkage and locating a disease locus, and it allows for exclusion mapping (Risch, 1990b, 1993). We observed MLS $\geqslant 1.0$ along an approximately 45-cM region of 6q (Fig. 1). Characteristic of complex disease mapping, the exact location of a candidate susceptibility locus could not be defined precisely; however, the maximum MLS was obtained at D6S454–D6S423. The MLS values are, as expected, higher when independence of the ASPs is assumed (whole sample: unweighted MLS = 3.04; weighted MLS = 1.7). In the subset of fully informative families (both parents genotyped), all MLS values in 6q were greater than 0.97. For this subset of families, the highest MLS value was also located at D6S454–D6S423 (unweighted MLS = 5.4 and weighted MLS = 3.4).

It has been shown that ignoring the dependence among sib pairs may lead to slightly liberal sharing χ^2 and MLS statistics, whereas the inclusion of a weighting function in the calculations causes the statistics to be too conservative, particularly when parental marker information is missing (Meunier *et al.*, in press). In multipoint MLS analysis, similar trends have

TABLE 3

Pairwise Nonparametric Linkage Results with the Sharing χ^2 Method (Terwilliger and Ott, 1993)

Chromosome position (cM)	Marker	*M*	%	un-wgt *P* value	*M*-wgt	%	wgt *P* value
13.6 (pterm.)	D6S296	145	43	—	109	42	—
17.1	D6S470	145	53	0.21	113	52	0.33
26.4	D6S259	114	52	0.36	93	49	—
29.2	D6S469	144	50	—	108	48	—
29.21	D6S289	129	52	0.33	99	51	0.41
29.22	SCA1	128	55	0.14	95	54	0.22
29.22	D6S260	150	52	0.3	114	51	0.40
30.6	D6S288	80	50	—	62	47	—
32	D6S274	130	57	0.06	108	56	0.12
33.7	D6S285	108	54	0.23	88	51	0.40
41.2	D6S461	129	51	0.39	96	49	—
41.21	D6S299	144	54	0.14	109	52	0.32
45.2	D6S306	92	52	0.31	77	50	—
45.8	D6S273	105	47	—	89	45	—
50.3	D6S291	100	47	—	85	44	—
61.5 (Centr.)	D6S426	142	53	0.23	107	51	0.39
75.4	D6S465	130	50	—	95	50	—
84.7	D6S455	121	52	0.34	91	51	0.44
91.6	D6S445	97	51	0.43	79	49	—
103.6	D6S424	100	58	0.052	71	57	0.13
111.2	**D6S301**	**118**	**64**	**0.0015**	**87**	**61**	**0.017**
117	D6S278	114	56	0.089	94	57	0.097
119	D6S404	102	58	0.056	82	61	**0.020**
119	**D6S416**	**84**	**69**	**0.00024**	**64**	**65**	**0.0097**
119	**D6S302**	**141**	**60**	**0.0072**	**107**	**58**	**0.0408**
119.9	**D6S474**	**126**	**64**	**0.0006**	**94**	**64**	**0.0035**
120.9	**D6S261**	**133**	**62**	**0.0024**	**102**	**59**	**0.031**
120.9	**D6S454**	**108**	**58**	**0.037**	**87**	**60**	**0.031**
120.9	**D6S423**	**98**	**63**	**0.0044**	**76**	**64**	**0.0074**
122.6	**D6S267**	**112**	**62**	**0.0079**	**73**	**55**	**0.012**
122.6	**D6S303**	**98**	**59**	**0.033**	86	**62**	**0.19**
126.7	D6S408	76	53	0.33	64	53	0.32
130.9	**D6S262**	**130**	**59**	**0.019**	**98**	**57**	**0.072**
133.6	D6S472	106	50	—	77	53	—
157.2	D6S290	130	49	—	96	50	—
163	**D6S415**	**122**	**59**	**0.026**	**91**	**60**	**0.027**
170.1	**D6S305**	**144**	**58**	**0.033**	**110**	**57**	**0.059**
193.6	D6S264	78	47	—	74	47	—
213.8	D6S297	83	45	—	75	42	—
218.9	D6S446	115	55	0.14	89	56	0.13
233.2 (qterm.)	D6S281	108	46	—	86	47	—

Note. M, number of shared alleles identical by descent (IBD) + nonshared. %, percentage of shared alleles. Un-wgt, independence of sib pairs is assumed for calculations; wgt, calculations are weighted for nonindependence (Hodge *et al.*, 1984). Comparisons for which $P < 0.05$ are shown in boldface. All *P* values in the table are nominal. We also computed the empirical *P* values for the markers having the highest significance levels for the two-point test: D6S416 empirical $P = 0.0005$, D6S474 empirical $P = 0.0045$.

been shown (Daly and Lander, 1996). Within the 6p area, including the previously reported linked area at 6p24–p22 and the HLA region (Schwab *et al.*, 1995; Straub *et al.*, 1995), all MLS values were less than 0.6.

We tested the likelihood of a chromosome 6 schizophrenia susceptibility gene in our genome scanning data set by computing maximum likelihood scores under a fixed λ_s (sibling risk ratio attributable to this putative schizophrenia gene, so that $Z_0 = 0.25/\lambda_s$ value). We used two different fixed values of λ_s: one for a locus of $\lambda_s = 3$ and the other for a locus of smaller effect $\lambda_s = 2$. Chromosome 6p was essentially excluded for $\lambda_s = 3$. However, only a small fraction of 6p could be excluded for a $\lambda_s = 2$ (Fig. 1). The MLS values for the 6p markers D6S296, D6S260, D6S274, and D6S285 were −5.53, −2.38, −0.90, and −0.70, respectively.

We evaluated the 6q region in a replication data set composed of families from the NIMH Schizophrenia Genetics Initiative with 14 markers from 6q13–q26. Linkage results by the sharing method are given for each marker (Table 4). Markers over a region of approximately 21 cM showed *P* values < 0.05 with analysis. The strongest evidence was obtained at D6S424 (IBD percentage = 64, $P = 0.0004$), at D6S423 (IBD percentage = 63, $P = 0.0009$), and at D6S283 (IBD percentage = 62, $P = 0.0009$). When a weighting function was applied (Hodge, 1984), the results remained positive (all families included: D6S283, IBD percentage = 62,

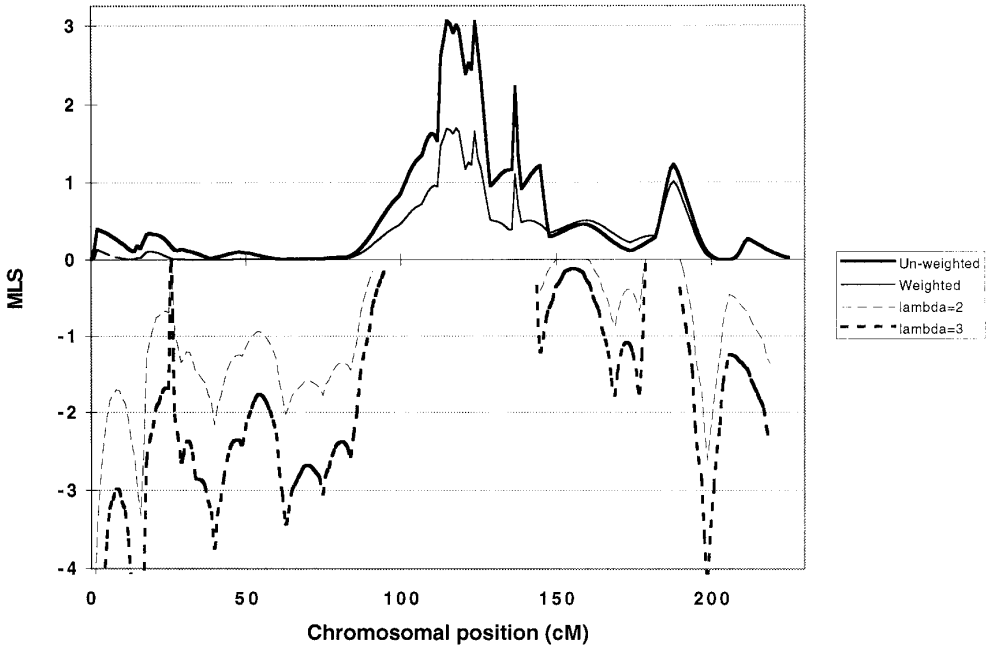

FIG. 1. Multipoint MLS scores (MLS expressed as $\log_{10}$ of the statistic) for chromosome 6 (Risch, 1990b) for the genome scanning data set (first data set). The exclusion maps are plotted for values 0 to −4.

P = 0.0027; and D6S423, IBD percentage = 62, P = 0.0045). Two-point MLS results (data not shown) were in reasonable agreement with the results obtained with the χ^2 sharing method. The highest MLS values were obtained at D6S424 (MLS 2.8, P = 0.00032) and D6S423 (MLS 2.64, P = 0.00047); both results support linkage. The two-point MLS values, as expected, are reduced when a weighting function is employed (D6S424, MLS 2.07, P = 0.0018; D6S423, MLS 1.78, P = 0.0037; and D6S283, MLS 1.96, P = 0.0024). Multipoint MLS values $\geqslant$1.0 started at the interval D6S455–D6S275, approximately 10 cM centromeric to equivalent scores in the first data set, and encompass 40 cM reaching the interval D6S267–D6S408 (Fig. 2). The maximum MLS of this data set is at the interval D6S300–D6S283, about 10 cM centromeric to the location of the maximum MLS of the genome scanning data set. Overall, these results support an overlapping area of linkage in 6q13–q26 in both data sets.

DISCUSSION

The results of this study provide suggestive evidence for linkage of schizophrenia to chromosome 6q in two independent data sets. The appropriate P values for declaring a linkage to be significant are not universally

TABLE 4

Pairwise Nonparametric Linkage Results with the Sharing χ^2 Method (Terwilliger and Ott, 1993)

Chromosome position (cM)	Marker	*M*	%	un-wgt *P* value	*M*-wgt	%	wgt *P* value
84.7	D6S455	176	52.1	0.2812	140	52.3	0.289
101.6	**D6S275**	**159**	**58.8**	**0.0133**	**130**	**57.7**	**0.038**
103.5	**D6S300**	**169**	**56.9**	**0.0385**	**132**	**57.8**	**0.034**
103.6	**D6S424**	**142**	**64.2**	**0.0004**	**112**	**63.4**	**0.002**
109.2	**D6S283**	**185**	**61.6**	**0.0009**	**149**	**61.5**	**0.003**
109.2	**D6S434**	**178**	**57.2**	**0.0263**	143	56.6	0.060
111.2	**D6S301**	**164**	**59.9**	**0.0051**	**128**	**60.6**	**0.008**
119.0	**D6S416**	**143**	**58.9**	**0.0154**	109	57.9	0.053
119.9	D6S474	173	55.8	0.0639	137	55.3	0.105
120.9	**D6S454**	**158**	**57.1**	**0.0386**	126	55.6	0.108
120.9	**D6S423**	**160**	**62.5**	**0.0009**	**124**	**61.8**	**0.005**
122.6	**D6S267**	**157**	**57.3**	**0.0342**	124	56.5	0.079
126.7	D6S408	136	53.8	0.1974	104	53.4	0.247

Note. M, number of shared alleles identical by descent (IBD) + nonshared. %, percentage of shared alleles. Unwgt, independence of sib pairs is assumed for calculations; wgt, calculations are weighted for nonindependence (Hodge *et al.*, 1984). Comparisons for which $P < 0.05$ are shown in boldface. All P values in the table are nominal.

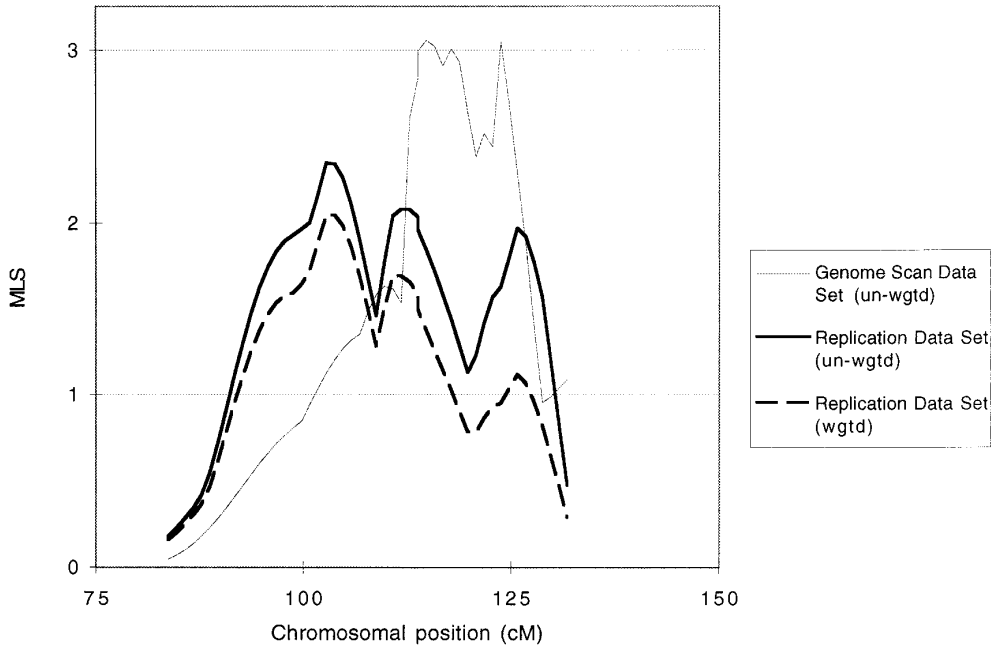

FIG. 2. Multipoint MLS scores (MLS expressed as $\log_{10}$ of the statistic) map for chromosome region 6q13–q21 for the replication data set (Risch, 1990b). Unweighted MLS values for the second data set are plotted in the background.

agreed upon. Lander and Kruglyak (1995) propose that a nominal P value of 2.2×10^{-5} is needed to declare linkage in a whole genome scan, using ASP analysis, and slightly less significant P values can be called suggestive linkage ($2.2 \times 10^{-5} < P < 7.4 \times 10^{-4}$). Our results with the genome scanning data set fulfill the "suggestive" criteria when either independence among ASPs is assumed for multipoint analysis or when empirical P values are calculated for the χ^2 sharing test (D6S416) (Table 3). Significance levels were lower when a weighting function that accounts for nonindependence among ASPs was used (Hodge, 1984). However, the inclusion of a weighting function for these calculations might cause the statistics to be too conservative and lose sensitivity, particularly when parental marker information is missing (Meunier *et al.*, in press). In our analyses, the use of a weighting function yielded larger P values than the computing of empirical P values (see Table 3). Notwithstanding, we would like to emphasize that these P values are guidelines only (Witte *et al.*, 1996) and that our conclusion from the initial data set is that this region of 6q may contain a schizophrenia susceptibility gene and needs to be studied in other data sets with markers in the 6q13–q21 area.

The replication data set also showed excess allele sharing on chromosome region 6q13–q26 markers, providing additional evidence for a susceptibility locus in 6q (Table 4). The highest sharing for this data set was at D6S424 (unweighted analysis, IBD percentage 64, $P = 0.0004$; weighted analysis IBD percentage: 63, $P = 0.0023$). Positive multipoint MLS scores show reasonable overlapping between the two data sets (Fig. 2). In our genome scanning data set, the multipoint MLS values were greater than 2.4 in the 11.4-cM interval delimited by D6S301 and D6S303, with a maximum value of 3.06 close to D6S278 and of 3.05 at D6S454/D6S423. In our replication data set >1 multipoint MLS values start approximately 10 cM centromeric to those in the genome scanning data set. The maximum MLS also occurs at a slightly more centromeric location than in the genome scanning data set. We would like to emphasize that in both data sets the area with MLS values $\geqslant 1$ greatly overlap. In contrast to our screening data set, the significance of linkage of our replication data set for the 6q13–q26 area does not require adjustment for multiple testing with hundreds of markers covering the whole genome. Therefore our results provide evidence for linkage of a schizophrenia susceptibility locus located on chromosome 6q at a significance level of $P < 0.001$.

There are some phenotypic and ethnic differences between the two data sets. The genome scanning data set includes, in addition to subjects with schizophrenia and schizoaffective, depressive-type disorder, individuals with schizoaffective disorder of bipolar type. In fact, of all ASP combinations, in 41.3% of the cases there is a subject with schizoaffective, bipolar-type disorder. Conversely, in the replication data set, families including siblings with this diagnosis were excluded from the initial collection. There are also differences in the ethnic make-up of these two samples, particularly regarding the proportion of families with predominant African-American ancestry: In the genome scanning data set, only 3.5% of the families were classified as African-American, while in the replication data set 35% of the families were of African-American ancestry. Interestingly, in spite of these differences, an overlapping area

of excess allele sharing and of positive two-point and multipoint MLS values was delineated. This indicates that a locus in chromosome region 6q13–q21, perhaps present in more than one ethnic group, may increase susceptibility for chronic psychosis, within the diagnostic boundary of DSM-III R schizophrenia, schizoaffective disorder, depressive type, and schizoaffective disorder, bipolar type.

Interestingly, two chromosomal abnormalities in chromosome 6q13–q26 have been described. Holland and Gosden (1990) reported psychotic illness associated in a three-generation family, with a pattern of dominant inheritance, with a balanced 6;11 chromosomal translocation with a breakpoint at 6q14.2, which is in our linkage area. In another report, a patient with acute paranoid psychosis was found to have a balanced 5;6 chromosomal translocation (Axelsson and Wahlstrom, 1984) with a breakpoint at 6q15, also within our linkage area. It is possible that these translocation breakpoints may alter the expression or function of the putative psychosis susceptibility locus in the region.

We have also examined for linkage in the region of chromosome 6p that was previously implicated in schizophrenia susceptibility (Straub *et al.*, 1995; Wang *et al.*, 1995). Attempts to replicate this observation have resulted in varied outcomes: several independent groups produced data that can be construed as providing a variable degree of support for this linkage (Moises *et al.*, 1995; Schwab *et al.*, 1995) while others have not (Gurling *et al.*, 1995; Mowry *et al.*, 1995). Our current results do not confirm this linkage either. However, the results of a combined analysis that included both previously published pedigree series and new series (including some of our families, which did not contribute to the linkage evidence) provided results supporting this linkage (Schizophrenia Collaborative Linkage Group for Chromosomes 3,6, and 8, 1997). The results that we report in this article are not, however, inconsistent with the existence of a 6p locus with $\lambda_s < 3$. In support of this argument of multiple susceptibility genes on one chromosome, one locus in 6p and two distinct loci (in two independent regions) on 6q have been reported in insulin-dependent diabetes mellitus (Davies *et al.*, 1994; Luo *et al.*, 1995).

In summary, while no linkage to an area of chromosome 6p was detected, our study shows very suggestive evidence for a locus on 6q in two independent data sets. We plan to follow up this study by fine mapping the linkage region, analyzing candidate genes in the region, and continuing the effort to recruit additional ASPs.

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