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Exclusion of a Schizophrenia Susceptibility Gene from the Chromosome 5q11-q13 Region: New Data and a Reanalysis of Previous Reports

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Summary

The report of a putative schizophrenia susceptibility gene linked to markers in the chromosome 5q11-q13 region and subsequent failures of replication have provoked considerable controversy. We here report six Welsh families multiply affected with schizophrenia in which there is no evidence for linkage between a dominant-like schizophrenia gene and 5q11-q13 markers. It is argued that our new results together with a combined reanalysis of previous studies suggest that a schizophrenia susceptibility gene can be excluded from the 5q11-q13 region. The apparent disparities between published results are most likely to reflect a chance finding in the one positive study and probably should not be interpreted as resulting from true linkage heterogeneity.

Introduction

There is compelling evidence, from family, twin, and adoption studies, of an important genetic contribution to schizophrenia (Gottesman and Shields 1982). Moreover, the results of classic twin and adoption studies based on traditional, descriptive clinical criteria have stood up to scrutiny when the data have been reassessed using modern, stricter, and more explicit research criteria (Kendler et al. 1981; McGuffin et al. 1984; Farmer et al. 1987). Although there is strong evidence from recent quantitative analyses that genes account for most of the variance in liability to schizophrenia (McGue et al. 1985), the mode (or modes) of transmission remains obscure. It seems unlikely that a single major locus, which is the sole source of resemblance between relatives, can account for the transmission of schizophrenia as a whole (O'Rourke et al. 1982; McGue et al. 1985), but the possibility remains that there is a gene of major effect, or several different major genes, which, together with polygenic or multifactorial environmental factors, contribute to the familiality of schizophrenia.

On the assumption that major gene effects may exist, several studies have been carried out using classical genetic markers (McGuffin and Sturt 1986). Promising preliminary results came from a study of HLA types in families multiply affected by a broadly defined clinical phenotype, "schizotaxia," consisting of schizophrenia plus schizotypal personality (Turner 1979). Analyses were performed under the assumption of a simple autosomal dominant mode of transmission and resulted in a maximum lod score of 2.57 at a recombination fraction of .15. However, four subsequent studies not only failed to replicate this finding but were able to effectively exclude linkage between a dominant gene for schizophrenia and HLA up to a recombination factor of .25 (McGuffin et al. 1983; Chadda et al. 1986; Andrew et al. 1987; Goldin et al. 1987; McGuffin 1988). Two of these studies reported data on a variety of other classical markers including blood group types, serum protein polymorphisms, and red cell enzymes (McGuffin et al. 1983; Andrew et al. 1987). Taken together, these data would allow exclusion of a dominant gene for

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schizophrenia from only about 6% of the genome (McGuffin 1988).

Thus, until very recently, genetic linkage marker studies in schizophrenia either followed up on a hint of linkage from a previous study or adopted a "random search" approach using whatever classical markers were available. The advent of a new generation of many DNA markers, RFLPs (Botstein et al. 1980) made possible by recombinant DNA technology, has led to the production of a nearly complete linkage map, albeit of low resolution, of the human genome (Donis-Keller et al. 1987). This has made it possible either to embark on a systematic search of the genome or to follow up on more specific clues from other sources, e.g., the association of chromosomal anomalies with schizophrenia. It was this latter strategy which led to a focus on the long arm of chromosome 5. Bassett et al. (1988) reported a Canadian family of oriental origin in which a young schizophrenic and his maternal uncle who was also schizophrenic were found to have a partial 5q trisomy. The young man's nonschizophrenic mother was a balanced carrier of the anomaly, but no other members of the family had either chromosomal abnormalities or schizophrenia. Subsequently, linkage between a putative schizophrenia susceptibility gene and markers in the 5q11-q13 region was reported. These positive findings were based on the study of two British and five Icelandic pedigrees. Multipoint analysis gave a maximum lod score of 6.49 for a dominant-like gene of high penetrance between the markers p105-599ha and p105-153ra (Sherrington et al. 1988).

Simultaneously, a report of failure to find linkage in this region was published based on an investigation of a large Swedish kindred (Kennedy et al. 1988). However, since the Swedish sample formed part of a relatively genetically isolated community, the possibility of linkage heterogeneity was raised. Two further negative reports then followed, one based on 15 Scottish pedigrees (St. Clair et al. 1989) and the other on five families from the United States (Detera-Wadleigh et al. 1989). The controversy occurring in the aftermath of these reports has hung on several issues. First it was suggested that the British-Icelandic and the Scottish findings were not comparable because of diagnostic methods and the methods by which the samples were obtained (Gurling 1990). In particular, it was suggested that failure to detect linkage may have been related to the fact that some of the Scottish pedigrees contained cases of both schizophrenia and manic-depressive illness and that both diagnoses were scored, for the purpose of linkage analysis, as "affected." Doubts have also been present about comparability of methods of analysis, since the positive British-Icelandic report explored a variety of single-gene models and included a variety of nonpsychotic "fringe" phenotypes as affected. Finally, some more minor difficulties in the published accounts emerge on close scrutiny. For example, the Scottish study contained inconsistent marker data in one pedigree (St. Clair et al. 1989), while in the British-Icelandic study the raw data on one family were completely omitted (Sherrington et al. 1988). Furthermore, in both cases the investigators analyzed the data by assuming a gene frequency which would give a population lifetime risk of schizophrenia well in excess of the usually quoted figure of about 1% (Gottesman and Shields 1982).

The purpose of the present paper is to report our findings on six families from south Wales multiply affected by schizophrenia and to combine our results with a reanalysis of previously published data. Our six pedigrees were selected because of lack of ambiguity about the diagnosis and, in particular, because of absence of any members with bipolar affective illness. In addition, the families were chosen as ones for which we could be reasonably certain about the "laterality" of the disorder, i.e., through which parental line the putative major gene for schizophrenia has been transmitted. We have set out to analyze pedigrees for evidence of such a gene in linkage with markers in the 5q11-q13 region, by using a model which, with minor modifications—such as a more realistic gene frequency (see below)—resembles the model which previously gave maximum evidence of linkage in the study of British and Icelandic pedigrees (Sherrington et al. 1988). In the hope of providing further clarification and resolving some of the more contentious aspects of the current debate, we then present a reanalysis of the already published data sets, applying the same model of transmission of schizophrenia. Finally we will compare the results of our reanalysis across the data sets and perform a formal test of heterogeneity.

Methods

Ascertainment and Diagnosis

Psychiatrists in South Wales were asked to refer for the study any schizophrenic patients who had at least one known living first-degree relative with the same diagnosis. Of these, the patients' families were selected for the study if they appeared to be potentially informative for linkage. That is, at a minimum the families consisted of parents and two or more offspring. One or more informants were contacted in each family, and

information was collected on second- and first-degree and more distant classes of relative. Subsequently the study of each pedigree was extended as far as possible to include all sibships in whom the disorder was known to be segregating. An attempt was made to preferentially select large to moderately large pedigrees, and for the purposes of the present report we focussed on families in which the clinical phenotypes were satisfactorily clear and, in particular in which there was no 'contamination' with manic-depressive illness of the bipolar type. Although selecting such loaded pedigrees is unlikely to represent the generality of schizophrenics' families and although the resulting Mendelian-like appearance can be misleading (Sturt and McGuffin 1985), one would expect that no bias with respect to detection of linkage would be introduced (Ott 1985).

All available family members were interviewed using a lifetime version of the Present State Examination (PSE) (Wing et al. 1974; McGuffin et al. 1986). This was augmented with some additional questions to allow diagnoses to be made according to DSM-IIIR criteria (American Psychiatric Association 1987). In addition, a checklist for schizotypal features was used which was a modification of the approach adopted in an earlier study (McGuffin et al. 1983). However, for the purpose of the present report, only subjects with definite current psychiatric illness or a past history of definite illness were considered as affected. In addition, where written material, such as hospital case notes, was available, this was scored on an Operational Criteria (OPCRIT) checklist for psychotic illness (a checklist which, again, is an extension of an earlier schedule [McGuffin et al. 1984]) to enable DSM-IIIR and other sets of operational diagnostic criteria to be made. In the present report on the Welsh pedigrees, subjects are classed as affected if they have schizophrenia, schizophreniform disorder, delusional disorder, or depressive psychosis with mood-incongruent delusions.

DNA Probe Hybridization

DNA was prepared from frozen and fresh blood of each available family member by standard methods (Old and Higgs 1983). For each restriction-enzyme analysis, 5 μg DNA was digested overnight with 10 units of restriction enzyme under conditions recommended by the manufacturer (Gibco BRL). DNA digests were fractionated by electrophoresis in 0.8%–1.2% agarose gels and were transferred to nylon filters (Hybond N; Amersham) in 20 × SSC (Southern 1975). Probes were radioactively labeled with ^{32}P by the random primer method (Feinberg and Vogelstein 1983) to a specific activity of 10^8–10^9 dpm/μg. Hybridization buffer contained 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, 20 mg herring sperm DNA/ml, and 20–100 ng radioactive probe. Hybridization was performed at 65°C for 16 h, followed by washing in 2 × SSC for 15 min twice, in 2 × SSC containing 0.1% SDS for 30 min, and in 0.1 × SSC for 10 minutes, all washes occurring at 65°C. Autoradiographs were exposed at −70°C with intensifying screens for 2–6 d.

Probe Details

The probe p105-599ha was used to detect three *Taq*I alleles of sizes 17, 14, and 10 kb, shown as 1, 2 and 3, respectively (allele frequencies .32, .16, and .52, respectively). To quench repetitive sequence hybridization, the labeled probe was preincubated with sonicated, denatured human DNA (Litt and White 1985). Probe p105-153ra hybridized to *Xba*I alleles of size 8.7 and 5.8 kb, shown as 1 and 2, respectively (allele frequencies .29 and .71, respectively), and probe p105-798rb hybridized to *Msp*I alleles of sizes 2.3 and 1.8 kb, shown as 1 and 2, respectively (allele frequencies .57 and .43, respectively).

Linkage Analysis

Two-point and multipoint linkage analysis was carried out using the LINKAGE package (Lathrop et al. 1984) in its recent version (version 5.03). Genotypic penetrances values were taken from the model of schizophrenia of Sherrington et al. (1988), which gave the strongest evidence for linkage. However, the gene frequency was altered to a more realistic value which was in keeping with the estimated population risk for schizophrenia. Thus the analyses were carried out under the assumption of a mutant gene A_2 with a frequency of .005 and a normal allele A_1 such that the penetrance (f_1) for the A_1A_1 genotype (i.e., sporadic cases) was taken as .001 while the penetrance (f_2) for the A_1A_2 genotype was taken to be .85 and the penetrance (f_3) for A_2A_2 was set at 1.0. Under the assumption of Hardy-Weinberg equilibrium, these figures correspond to a population frequency, K_p, of 0.86%, which is the estimated population morbidity risk for schizophrenia in Britain, as based on data from the Camberwell register maintained in London (Gottesman and Shields 1982). This is given by the expression $K_p = f_1(1-q)^2 + 2f_2(1-q)q + f_3q^2$. For all analyses it was assumed that the female:male recombination ratio was 3:2. So as to allow comparability with the positive results of Sherrington et al. (1988), in which all individuals

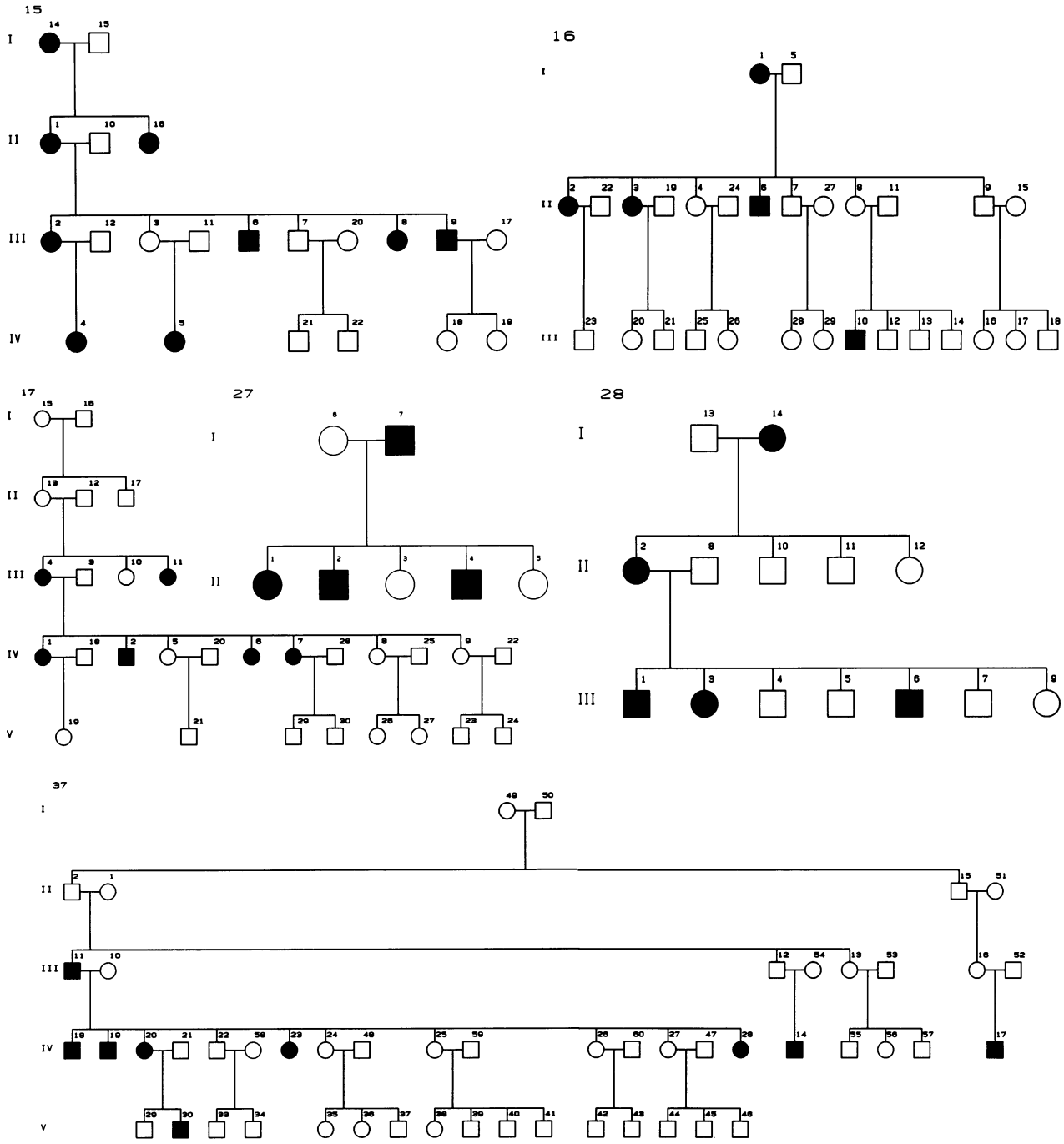

Figure 1 Six pedigrees multiply affected by schizophrenia (black symbols denote affected individuals; see text and table 1 for definition of phenotype).

were considered to belong to a single liability class, no age-at-onset correction was applied.

Heterogeneity

Heterogeneity analysis was carried out using the admixture test of Smith (1963), as implemented in the HOMOG program of Ott (1985). We used the most recent PASCAL version (version 2.51) of HOMOG, with modifications by L. Sandkuyl (personal communication) which allow analyses to be performed on multipoint data.

Table 1

Diagnosis, Age, and Marker Genotypes

Pedigree and Individual	Diagnosis[a]	Age (years)	RFLP Allele		
			P105-599	P105-153	P105-798
15:					
2	1	56	1 3	2 2	1 2
3	0	59	1 3	1 1	1 2
4	1	27	1 3	2 2	1 2
5	1	32	1 3	1 1	1 1
8	1	54	1 3	1 2	1 1
11	0	58	1 3	1 2	1 1
16:					
1	1	70	1 3	1 2	1 2
2	1	46	1 1	1 2	1 1
3	1	40	1 1	1 2	1 2
4	0	35	1 3	1 2	1 2
5	0	68	1 1	1 2	1 1
6	1	27	1 3	1 2	1 2
7	0	30	1 1	1 2	1 1
8	0	38	1 3	1 2	1 2
9	0	44	1 3	1 2	1 2
10	1	18	1 2	1 1	1 1
17:					
1	1	30	3 3	1 2	1 1
2	1	20	1 3	1 2	1 2
3	0	60	1 3	2 2	1 1
4	4	58	2 3	1 2	1 1
5	0	32	1 3	1 2	1 1
6	1	26	1 3	1 2	1 1
7	3	36	1 3	2 2	1 1
27:					
1	1	35	2 3	1 2	1 1
2	1	30	2 3	1 2	1 1
3	0	33	1 3	1 1	1 1
4	2	37	1 3	1 2	1 2
5	0	23	1 3	1 2	1 1
6	0	59	2 3	1 2	1 2
7	3	65	3 3	1 2	1 2
28:					
1	1	38	2 3	1 2	1 2
2	1	54	1 3	1 1	1 1
3	1	28	2 3	1 2	1 2
4	0	36	1 3	1 2	1 1
6	1	26	2 3	1 2	1 2
8	0	60	2 3	1 2	1 2
10	0	46	1 3	1 2	1 1
11	0	41	3 3	1 2	1 2
12	0	36	3 3	1 2	1 2
37:					
10	0	75	1 3	1 2	1 1
11	3	74	2 3	1 2	1 2
18	1	50	3 3	2 2	1 2
20	1	47	1 3	1 2	1 2
22	0	44	1 3	2 2	1 2
26	0	32	1 2	1 2	1 2
28	1	21	1 2	2 2	1 2
29	0	27	1 1	1 1	1 1

[a] From DSM-IIIR. 0 = no diagnosis; 1 = schizophrenia; 2 = schizophreniform disorder; 3 = delusional disorder; 4 = major depression with psychosis.

Table 2

Two-Point Lod Scores

Probe	Recombination Fraction					
	0	.1	.2	.3	.4	.5
p10-599ha	−9.294	−1.792	−.700	−.201	−.003	.0
p10-153ra.....	−.993	−.195	−.025	−.010	−.005	.0
p10-798rb.....	−3.540	−1.547	−.693	−.276	−.074	.0

Results

A. South Wales Pedigrees

The pedigrees are illustrated in figure 1, and marker genotypes are listed in table 1. Two-point lod score analysis (table 2) enables us to exclude tight linkage with p105-599ha and p105-798rb; and, with both these markers, linkage up to recombination fraction of .1 is unlikely. The two-point data concerning p105-153ra are less informative. However, when we carried out multipoint analysis with schizophrenia as the test locus, with p105-599ha arbitrarily fixed at 0 cM, p105-153ra fixed at 12 cM, and p105-798rb fixed at 20 cM (Leppert et al. 1987), we obtained negative lod scores throughout this entire region of chromosome 5q (fig. 2). Our data are therefore consistent with the three previously published negative studies (Kennedy et al. 1988; Detera-Wadleigh et al. 1989; St. Clair et al. 1989) and are at variance with that of Sherrington et al. (1988). We therefore proceeded to a reanalysis of the previously published data so as to compare and combine them with our own.

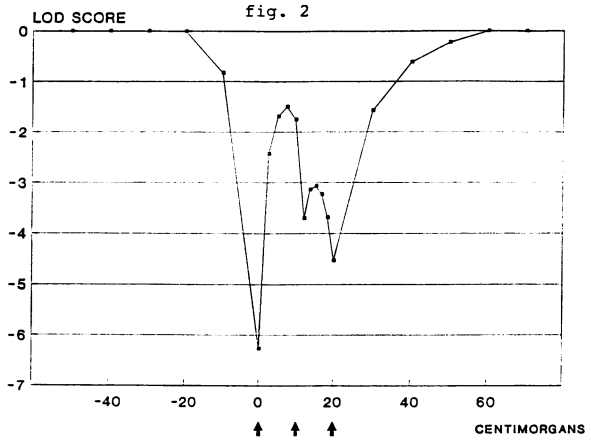

Figure 2 Multipoint lod score analysis with schizophrenia as test locus against fixed points (arrows), where p105-599ha is at 0, p105-153ra is at 12 cM, and p105-798rb is at 20 cM.

B. Combined Analysis

Results of multipoint analyses are summarized in table 3. The analyses were performed under the assumption of a near dominant model of transmission of schizophrenia, as outlined above. Where the published data allowed more than one alternative definition of the affected phenotype, the broadest definition was used, since this strategy was the one which resulted in the strongest evidence of linkage in the positive study (Sherrington et al. 1988). Inconsistencies in published marker genotypes were corrected (D. St. Clair, personal communication), and the missing pedigree from the published report on the British-Icelandic study was supplied by R. Sherrington and H. Gurling (personal communication). So as to preserve anonymity, Sherrington et al. (1988) "disguised" their pedigrees, including altering the sex of some individuals. However, these authors reported that the changes as presented in their published paper had minimal effects on the lod scores. We therefore analyzed these pedigrees as published (and indeed obtained results very closely similar to those reported by Sherrington et al. in their analysis of the nondisguised pedigrees).

All published studies provide information on p105-599ha and p105-153ra, and a multipoint analysis was carried out with these markers fixed at 0 and 12 cM, respectively, with both schizophrenia as the test locus and the same single-gene model parameters once again used. The results of this are shown in table 3. A graphic plot of the combined data analysis is shown in figure 3. Slightly positive lod scores were obtained on the centromeric side of p105-599Ha, and the scores rose to a maximum of 2.6 on the telomeric side of p105-153ra (table 3) when data from both the present study and all four published studies were combined; but, as can be seen in figure 3, the positive scores appear to be entirely due to the study of Sherrington et al. (1988), and they disappear when the British-Icelandic data are removed.

Nevertheless, the hint of positive lod scores telomeric

Table 3

Three-Point Linkage Analysis, by Study

Study	Map Distance (cM)													
	−40	−30	−20	−10	.0[a] (p105-599Ha)	2.4	4.8	7.2	9.6	12[a] (p105-153Ra)	22	32	42	52
Present study	.039	.005	−.272	−1.232	−8.587	−3.133	−1.774	−1.038	−.642	−.790	.361	.440	.278	.096
St. Clair et al. 1989	−.067	−.377	−1.345	−4.227	−19.615	−11.356	−9.354	−8.519	−8.655	−12.617	−3.531	−1.195	−.359	−.074
Kennedy et al. 1988	−.014	−.064	−.169	−.346	−.552	−.529	−.501	−.471	−.446	−.427	−.190	−.078	−.027	−.005
Detera-Wadleigh et al. 1989	−.037	−.061	−.439	−1.534	−9.097	−4.345	−3.418	−3.037	−2.999	−3.359	−.967	−.327	−.044	−.042
Sherrington et al. 1988	.561	2.182	4.072	5.297	−.614	4.499	5.550	6.036	6.192	5.678	5.524	3.770	1.843	.433
Combined:														
Excluding Sherrington et al. data	−.079	−.497	−2.225	−7.339	−37.851	−19.363	−15.047	−13.065	−12.742	−17.193	−4.327	−1.160	−.152	−.025
Including Sherrington et al. data	.482	1.685	1.847	−2.042	−38.465	−14.864	−9.497	−7.029	−6.550	−11.515	1.197	2.610	1.691	.408

Note.—An autosomal dominant model of transmission was assumed, with disease gene frequency of .005 calculated from estimated population prevalence of 0.86% and with phenocopy penetrance of .001, heterozygote penetrance of .86, and disease homozygote penetrance of 1.00 being assumed for each of the published studies.

[a] For male recombination rates.

fig. 3

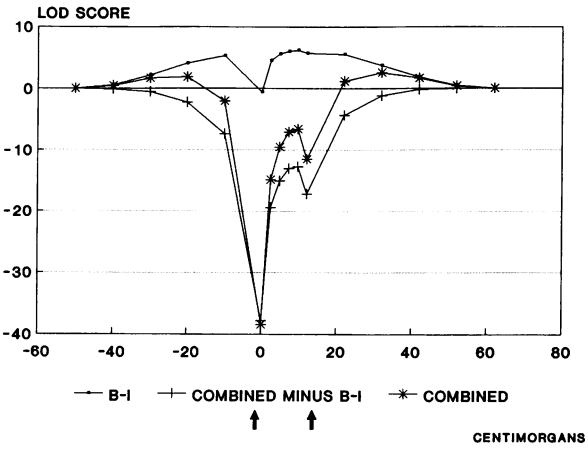

Figure 3 Multipoint lod score analysis of schizophrenia as test locus against fixed point at 0 (p105-599ha) and 12 cM (p105-153ra). Combined data are from the present study and four published studies. (Kennedy et al. 1988; Sherrington et al. 1988; Detera-Wadleigh et al. 1989; St. Clair et al. 1989). "BI" denotes the British-Icelandic study of Sherrington et al.; "Combined minus B-I" denotes the combined data with the data of Sherrington et al. removed.

fig. 4

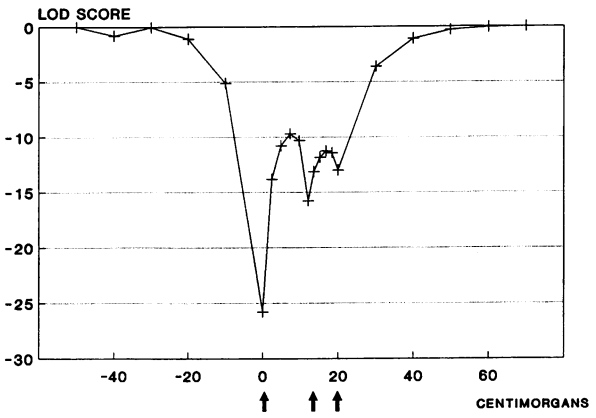

Figure 4 Combined multipoint lod score analysis of three data sets from the present study and those of Kennedy et al. (1988) and St. Clair et al. (1989). Schizophrenia is the test locus against fixed points (arrows), as in fig. 2.

of p105-153ra makes it particularly useful to have further marker information in this area. An RFLP detected by using the probe p105-798rb is located on the telomeric side of p105-153ra about 20 cM distant from p105-599ha. Only two published studies (Kennedy et al. 1988; St. Clair et al. 1989) provided data on p105-798rb, and these data were used in a four-point analysis once again applying the same single-gene model parameters and fixed locus distances that were used in the analysis of our south Wales pedigrees. Results of these analyses are shown graphically in figure 4. It is clear both from figures 3 and 4 and from the results given in table 3 that combining the data from the five different studies would strongly suggest that a gene for schizophrenia can be excluded from that entire region of 5q11-q13 where linkage has previously been reported. However, it is also clear that there is a marked disparity between the Sherrington et al. (1988) findings and those from the other four centers.

C. Heterogeneity

Results from heterogeneity testing are summarized in table 4. Two analyses were performed. In the first, all of the data from the present study plus those from the four other centers were analyzed. So as to have the best possible comparability, in testing for heterogeneity we used only the results of three-point analysis (i.e., multipoint lod score with schizophrenia as the test locus and with p105-599ha and p105-153ra fixed). The hypothesis H2, i.e., linkage in a proportion of cases (35%), was strongly favored over the H0 hypothesis, i.e., no linkage. The H1 hypothesis i.e., linkage and homogeneity, was also favored over the H0 hypothesis (table 4). However, as noted above, the entire evidence for linkage appears to come from just one study where all pedigrees support the hypothesis of linkage, whereas in the other studies the data are uniformly unsupportive of this hypothesis. We therefore performed a second analysis of the data, by removing the results for the British-Icelandic study. When this is done, there is no remaining evidence of heterogeneity, and we can conclude that the data do not allow rejection of the H0 hypothesis.

Discussion

In our study of Welsh multiply affected pedigrees we have failed to find evidence of linkage between a dominant-like schizophrenia susceptibility gene and markers in the chromosome 5q11-q13 region. Our analyses were carried out under assumptions about the mode of transmission for schizophrenia which, with minor alterations including a more realistic gene frequency, were close to those of the model which provided the

Table 4

Analysis of Heterogeneity for Three-Point Data

	Combined Data					
	Including Data of Sherrington et al. 1988			Excluding Data of Sherrington et al. 1988		
	Max ln1	alpha[a]	theta[b]	Max ln1	alpha[a]	theta[b]
Hypotheses:						
H2 (heterogeneity)	7.7438	.35	12.0000	.133	.1	52.00
H1	6.0086	(1)	14.0000	.133	(1)	52.00
H0	(0)	(0)	(50.00)	(0)	(0)	(50.00)
Components of χ^2:						
H2 vs. H1 (heterogeneity)	1	3.47	.0312	.00	.5	
H1 vs. H0 (linkage)	2	15.488	.0002	.266	.438	

[a] Proportion of linked cases.

[b] Distances (in cM) of test locus from arbitrarily fixed zero at p105-599ha.

maximum evidence for linkage in a previous study (Sherrington et al. 1988). Furthermore, when we combined our data with those from four previously published studies (Kennedy et al. 1988; Sherrington et al. 1988; Detera-Wadleigh et al. 1989; St. Clair et al. 1989), there was strong evidence against linkage, with large negative lod scores throughout the region between markers p105-599ha and p105-153ra, where a putative schizophrenia susceptibility locus has been claimed. Slightly positive lod scores were found just outside this region, on the centromeric side of p105-599ha, but, more particularly, on the telomeric side of p105-153ra. However, these appeared to be contributed entirely by the study of Sherrington et al. (1988) and disappeared when the data from this study were removed from the analysis. Nevertheless, it might still be argued that there is a susceptibility locus for schizophrenia telomeric of p105-153ra—rather than between p105-599ha and p105-153ra, as previously reported. It is therefore of value that both in the present study and in two of the previous reports (Kennedy et al. 1988; St. Clair et al. 1989) we have information on a third marker, p105-798rb, which is located about 8 cM away from p105-153ra in a telomeric direction. Our own data provide lod scores of −3 or less throughout the region between p105-153ra and p105-798rb, and combining our results with those from the two other centers provides convincing exclusion of a schizophrenia gene in this region. Moreover, the results are in keeping with those of Detera-Wadleigh et al. (1989), who reported data on a fourth marker, dihydrofolate reductase (DHFR), which is telomeric of p105-798rb.

How then can we account for these disparate findings—i.e., a strongly positive result in one study based on a mixture of British and Icelandic pedigrees and two negative results from elsewhere in the United Kingdom, (the St. Clair et al. [1989] study in Scotland and the present study in Wales), together with negative results in a Swedish pedigree (Kennedy et al. 1988) and in North American pedigrees (Detera-Wadleigh et al. 1989)? Broadly, there are three possible explanations. First, the results may reflect true genetic heterogeneity within schizophrenia, such that Sherrington et al. (1988) discovered seven families in which schizophrenia is transmitted via a gene on chromosome 5q while other groups have been studying a genetically distinct type (or types) of disorder. Second, there may have been systematic errors giving a false appearance of linkage in the positive study or obscuring evidence of linkage both in the present study and in the three other negative ones. Third, the single positive study may represent a chance finding. We will examine each of these possibilities in turn.

At first sight the heterogeneity hypothesis has some appeal. The positive study of Sherrington et al. (1988) was based on five Icelandic and two British pedigrees, and it could be argued that Iceland is comparatively genetically isolated and that there may be a schizophrenia gene which is segregating there but which is rare in other parts of the world. However, positive results were also obtained in the two British families, a result which is in contrast to the almost uniformly negative findings both in the Scottish study of St. Clair et al. and in our own study. When an admixture test is performed, there is highly significant evidence of hetero-

geneity within the results from the 34 families contributed by the five studies. However, all of the evidence for heterogeneity comes from the one positive British-Icelandic study, and there is no evidence of heterogeneity when the remainder of the material is analyzed with these seven pedigrees removed. Therefore we consider that true heterogeneity is unlikely to exist, because it would be expected that at least some suggestion of heterogeneity would be present when all of the negative studies were combined. In other words, with the exception of the Swedish pedigree by Kennedy et al. (1988), other published reports, have like our own study, consisted of several moderately large pedigrees rather than of a small number of very large kindreds. Therefore, we would expect there to be a reasonable probability of at least a minority of these families containing the chromosome 5q–linked form of schizophrenia. However, there does not appear to be even a hint of such an admixture.

We next need to entertain the possibility of systematic error either in the one positive study or in both the present study and the other three negative reports. The most obvious source of systematic error would be if strict "blindness" were not maintained between those investigators performing the genetic marker analysis and those assigning psychiatric diagnosis. This is such a basic methodological point that it seems unlikely that it was overlooked in any of the studies. The second possibility is that division of samples could have occurred, leading to false inference of linkage in the positive study. Thus a large sample may show a suggestion of linkage in some families but not in others, and it might be tempting to focus on the "positive" families and set aside those which would not be compatible with linkage. A series of modestly positive scores, when summated, could then give a misleading and spurious appearance of linkage. This again seems an unlikely explanation for the disparate findings regarding chromosome 5q11-q13 and schizophrenia.

Last, we have to consider the possibility that the study of Sherrington et al. represented a chance positive finding. On the face of it this also seems unlikely, since multipoint analysis gave a maximum lod score in excess of 6. However, we have to consider whether multiple testing increased the probability of apparent linkage when in fact no linkage exists on 5q11-q13. In the reanalysis which we have carried out in the present paper we deliberately restricted ourselves to one model of transmission of schizophrenia, a model which was closely similar to the one giving the greatest evidence of linkage in the original report of Sherrington et al. (1988). We also restricted ourselves to one definition of the schizophrenia phenotype, again selecting, both in our own material and in the reanalyzed published material, a broad definition, on the basis that this should result in the strongest evidence for linkage if the report of Sherrington et al. (1988) was correct. However, in the study of Sherrington et al. several different ways of defining the schizophrenia phenotype were explored, and there was also an exploration of a range of models of transmission.

Employing multiple different definitions of the phenotype and multiple different possible models of transmission means that not just one test for linkage but many different tests were carried out on the same sample. The problem would be compounded if further tests were also carried out for markers at loci other than on chromosome 5q11-q13. The problem of multiple testing has long been recognized in connection with association studies (Weiner 1961). In testing for allelic association in multiple marker systems—or in systems where there are multiple alleles, such as the HLA system—it has become customary to adopt a probability level for significance tests that is more conservative than the conventional P value of .05 or less. A particularly conservative but commonly used correction is to multiply P values by the number of tests carried out (Svjegaard 1975). By contrast, the problem of multiple testing has so far received much less attention in linkage studies but probably deserves to receive more attention now that a very large number of polymorphisms are available (Ott 1985). The conventional criterion for acceptance of linkage is a lod score of 3 (Morton 1955). This corresponds to odds on linkage of 1,000:1, but since the prior probability of detecting linkage between two loci is low (it is of the order of .054 for two genes just being on the same chromosome), a lod score of 3 corresponds to a posterior probability or "reliability" of .95 (Morton 1955, 1982). This of course pertains only to two-point analysis where both the main trait and the marker locus have simple and well-established modes of transmission. When multipoint analysis has been carried out, and especially where the mode of transmission of the main trait is unknown, the situation becomes much less clear. One solution is that if there is to be multiple testing with different markers, combined with the effects of exploring different models of transmission and different definitions of the phenotype, linkage studies of diseases in schizophrenia should only be considered definitely positive if a very high lod score is achieved. The danger then is that we become overconservative and that the risk of type 2 errors be-

comes great. A pragmatic alternative is to retain the conventional lod score of 3 as indicating a "possible positive" which requires independent replication on a second sample before it can be regarded as a "probable positive."

We conclude that our own data plus our reanalysis of published material effectively exclude a gene for schizophrenia in the region of chromosome 5q11-q13, where such a gene was previously reported. This does not, of course, have any bearing on the older argument over whether there is a genetic basis for schizophrenia. We think that an important genetic contribution to schizophrenia certainly does exist, but the evidence for this comes not from linkage studies but from classical family, twin, and adoption investigations (Gottesman and Shields 1982). Nor do the negative linkage data discussed here invalidate the pursuit of genetic marker strategies in studying the genetic basis of schizophrenia. Despite the variety of methodological problems and potential practical difficulties which we have discussed, a systematic search throughout the human genome can now be justified as a means of detecting major gene effects in schizophrenia and will certainly be successful if such genes exist, i.e., if the genetic component of liability to develop schizophrenia is not entirely contributed by many genes of small effect.

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