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The Iowa Multiplex Family Study of Schizophrenia: Linkage Analyses on Chromosome 5*

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Summary. We analysed six multiplex pedigrees of schizophrenia for linkage to two DNA probes mapping to the chromosome 5q11–q13 region where linkage to a gene for schizophrenia was recently reported. Analyses were conducted using three penetrance models and considering the affected state to be schizophrenia, the schizophrenia spectrum, and all psychiatric diagnoses. All analyses gave consistently negative lod scores. Although the region was not formally excluded, no evidence for linkage was found.

Key words: Schizophrenia – Linkage – Genetics

Introduction

Schizophrenia is a devastating illness and one that has proved refractory to biological research until recently. Therefore, it is understandable that evidence of a gene on chromosome 5 predisposing to it has created considerable interest (Sherrington et al. 1988).

The discovery that first aroused interest in chromosome 5 was an inverted translocation of chromosome 5q11.2-q13.3 on chromosome 1 in a patient with schizophrenic symptoms and dysmorphic facial features (Bassett et al. 1988). The patient's maternal uncle also had schizophrenic symptoms, dysmorphic features and the same translocation. This is such a unique combination of features that it would have been informative to have had the full clinical picture reported. Since both schizophrenic relatives were triploid for 5q11.2–q13.3 (i.e., they had two normal chromosomes 5 plus the translocated region on chromosome 1), the finding suggested a gene in that region predisposing to schizophrenia.

Molecular genetics provides an efficient means of detecting disease genes through linkage to DNA probes, and fortunately, a number of probes in the region of interest are available. Sherrington et al. (1988) studied seven Icelandic and English schizophrenic pedigrees and reported linkage to two markers in the 5q11–q13 region. Unexpectedly, the strength of the finding increased as the definition of the schizophrenia spectrum was broadened to include all psychiatric illnesses. Kennedy et al. (1988) were unable to replicate the finding in pedigress from northern Sweden, suggesting that more than one gene for schizophrenia may exist. Obviously, the next task must be an attempt to replicate the linkage finding in pedigrees from other sources in order to verify and determine the frequency of the chromosome 5 linkage.

We have been collecting multiplex pedigrees of schizophrenia in Iowa as part of a family study of neuropsychiatric dysfunctions in schizophrenia. This paper reports our progress to date on the first six pedigress that are informative for linkage.

Methods

Probands. The probands are patients hospitalized at the Iowa Psychiatric Hospital who have been interviewed with the Comprehensive Assessment of Symptoms and History (CASH) (1987) interview and diagnosed as schizophrenia by concensus agreement of two project psychiatrists. Those probands with at least two family members diagnosed by family history as having a schizophrenia spectrum diagnosis are included in the pedigree study. The schizophrenia spectrum includes schizophrenia, schizoaffective disorder, schizophreniform disorder, and schizotypal personality disorder.

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Pedigrees. The probands and first-degree relatives are assessed with an instrument battery that includes the CASH (Andreasen 1987), Schedule for Schizotypal Personlities (Baron et al. 1981), Chapman scales (Chapman and Chapman 1980; Chapman et al. 1976, 1978), a neurological examination, assessments of attention and information processing, and magnetic resonance imaging (MRI) of the brain.

The pedigress are extended according to a modification of the Cannings and Thompson (1977) rule. The pedigree is extended through affected persons to include all first-degree relatives of that person. The modification is that an unaffected person with an affected first-degree relative counts as affected, allowing for the incomplete penetrance of schizophrenia. Extended family members receive the interview battery but not the neuropsychological assessments.

Diagnoses. Diagnoses are made according to DSM-IIIR by two project psychiatrists by concensus agreement after reviewing the interview material and medical records. For the purposes of the linkage analyses the affected phenotype is considered to include schizophrenia only, schizophrenia plus the spectrum as defined above, or any axis I diagnosis. These three classifications of the affected phenotype are similar to those used by Shertington et al. (1988). All other diagnoses are considered unaffected.

Laboratory Analyses. Blood samples are obtained from all family members and permanant lymphoblastoid cell lines are established (Neitzel 1986). DNA is extracted from the cells by phenol-chloroform, digested with restriction enzymes according to the protocol recommended by the supplier (BRL, Inc.) and electrophoresed in 0.8% agarose gels. The DNA is transferred to Zetabind nylon membranes (Cuno, Inc.) with 0.4 N NaOH. DNA probes are labeled with P32dCTP by the primer extension method (Feinberg and Vogelstein 1983) using a kit (Amersham, Inc.), hybridized to the membranes as recommended by the manufacturer (Cuno, Inc.). The membranes are washed at high stringency (0.1 × SSC, 0.1% SDS) to remove background radiation and exposed to Kodak XAR-5 film with Cronex lightening-plus image intensifier screens to produce autoradiographs.

Probes. Two probes were used in the present study: D5S39 and D5S76 (p105–153Ra and p105–599Ha obtained from the American Type Culture Collection, Rockville, md.). These are the two probes found to be linked to schizophrenia in the Sherrington et al. (1988) study. D5S39 detects a two allele polymorphism with MspI and another with XbaI, and the two can be haplotyped to yield a four allele system. D5S76 detects a three allele system with TaqI. The autoradiographs were scored for these allele systems without knowledge of diagnosis.

Linkage Analyses. The program LINKMAP of the LINKAGE package (Lathrop et al. 1985) was used to calculate lod scores for the region surrounding the two probes. The genetic distance between the two marker loci was arbitrarily set at 20 cM.

The genetic model assumed a disease gene frequency of 0.008, autosomal inheritance, and a penetrance of 0.7. In addition, a gene frequency of 0.01 and penetrances of 0.5 and 0.9 were tested.

Three classifications of the affected state were tested. The first included only schizophrenia as affected and all other diagnoses as unaffected. The second included schizophrenia plus the schizophrenia spectrum as defined above. The third set of analyses included these diagnoses plus affective disorder, the only axis I diagnoses outside of the schizophrenia spectrum.

Table 1. Lod scores for analyses of six schizophrenia pedigrees

Morgans	Schizophrenia	Schizophrenia spectrum	Schizophrenia spectrum depression
1.20	0.0000	0.0000	0.0000
1.10	−0.0014	−0.0161	0.0121
1.00	−0.0157	−0.0759	0.0368
0.90	−0.0756	−0.2176	0.0357
0.80	−0.2659	−0.5361	−0.0853
0.75	−0.5054	−0.8624	−0.2904
0.70 D5S39	−1.4430	−1.9073	−1.1970
0.66	−0.6849	−1.1475	−0.3534
0.62	−0.5787	−1.0441	−0.1607
0.58	−0.6707	−1.1601	−0.1551
0.54	−1.0427	−1.5609	−0.4056
0.50 D5S76	−4.2060	−4.7000	−3.4022
0.45	−1.4216	−1.8966	−0.7607
0.40	−0.8646	−1.2625	−0.3332
0.30	−0.3429	−0.5731	−0.0330
0.20	−0.1181	−0.2219	0.0235
0.10	−0.0247	−0.0511	0.0112
0.00	0.0000	0.0000	0.0000

Results

Thirty-five persons in six pedigrees were evaluated and genotyped. Eleven of these were diagnosed as schizophrenia, three as depression, two as schizotypal personality, and one each as schizoaffective and schizophreniform disorder. Linkage analyses were performed considering three classifications of these disorders affected as described in the Methods section. A lod score of 3.0 is required to confirm linkage and a lod score of −2.0 to exclude it. The results of the analyses are presented in Table 1, which shows lod scores across 120 cM of chromosome 5q11–q13. (One centimorgan represents a recombination fraction of 0.01, or about one million base-pairs of DNA).

Close linkage to D5S76 could be excluded for all three definitions of affected, and close linkage to D5S39 was unlikely, as the lod scores ranged from −1.2 to −1.9. The region between the two markers could not be formally excluded, but none of the lod scores was positive. Furthermore, when the schizophrenia spectrum was considered to be affected, the lod scores remained below −1 across the 20 cM bounded by the two markers.

Discussion

The analyses to date have not produced evidence in support of linkage between schizophrenia and

markers located at chromosome 5q11–q13. The failure to find evidence of linkage could have a number of reasons. In the first place, the number of pedigrees and the number of affected individuals is still relatively small. Since little information is obtained from unaffected individuals when penetrance is reduced, the lod scores were supported largely by the affected subjects.

The definition of the affected phenotype may have been too broad. Presently, there are only five schizophrenic family members in addition to the six probands, so an analysis based on strict schizophrenia was not very informative. However, it is notable that in the Sherrington et al. (1988) study the greatest lod scores were obtained with the broadest definition of the affected phenotype, and in the present study the addition of the schizophrenia spectrum to the affected class resulted in the lowest lod scores.

Considering both the Sherrington et al. (1988) and the Kennedy et al. (1988) results, it appears that schizophrenia is etiologically heterogeneous. In that case our six pedigrees could represent more than one genetic form, obscuring a single locus. Unfortunately, none of the pedigrees is large enough to determine whether individual pedigrees support linkage. Even if they were genetically homogeneous, they could represent a different genetic form of the disease from those that have supported linkage.

We excluded close linkage to D5S76 and consistently obtained a lod score of less than -1.0 at D5S39. Likewise, the Sherrington et al. (1988) analysis excluded close linkage to D5S76 and the lod score was lower at D5S39 than it was in the surrounding region, although that locus was not excluded. The most likely location of the disease gene was either between the two markers or on either side of them. Our results indicate that a disease gene for schizophrenia is unlikely to lie between the two or immediately centromeric to D5S76. However, the lod scores for linkage telomeric to D5S39 fell sharply toward zero with increasing gentic distance, so the presence of a disease gene in that region could not be tested.

Obviously, it would be helpful to know how much pedigree material would be needed to have a reasonable chance of finding linkage if it were present, or of excluding it if it were not. Formal power calculations for linkage analysis are possible and have recently been implemented for diseases with incomplete penetrance (Ploughman and Boehnke 1989). However, it is encouraging that rather strongly suggestive lod scores have been obtained with the limited amount of pedigree material that has been collected as this time.

The purpose of this report has been to illustrate the use of DNA markers in searching for disease genes for familial psychiatric disorders. The results underscore the need for large multiplex pedigrees so that more definitive lod scores can be obtained. Our plans are to continue extending these pedigrees as well as adding new ones. It is also important to assess the family members as broadly as possible so that all phenotypes can be used in the data analysis, when appropriate. Thus, data on possible trait markers such as eye movement abnormalities, and disturbances in attention and information processing may detect a linkage that would be missed if that information were not available. If disease genes are to be found in disorders as complex as schizophrenia, it will require a maximum of effort at both the clinical the genetic levels.

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