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DNA Markers and Biological Vulnerability Markers in Families Multiply Affected with Schizophrenia

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Summary. A family is reported in which the monozygotic co-twin of a schizophrenic proband was diagnosed bipolar 1 and their mother had a history of unipolar major depression. Although their clinical manifestations varied, the ill members of this family shared an abnormality in P300 not found in the asymptomatic siblings. In 14 families, linkage to the 5q11–13 region was excluded when affection status was defined solely by P300 latency inpendently of the clinical findings. Linkage was also excluded when the analysis was restricted to the families that had no cases of bipolar illness and when the schizophrenic phenotype was narrowly or broadly defined. It is concluded that biological markers such as P300 and eye tracking may help to clarify the overlap of different types of psychosis and help to define the phenotype for linkage analyses.

Key words: Schizophrenia – DNA markers – Linkage – P300 – Eye-movement disorder

Introduction

Uncertainties about the definition of the phenotype is a major weakness of linkage studies in schizophrenia, and the status of bipolar illness when diagnosed in first-degree relatives of probands is a particular source of controversy. It has been proposed that families in which both diagnoses occur should be excluded from analysis on the grounds that two separate genes may be segregating (Sherrington et al. 1988; Byerley 1989). However, this argument could equally well be applied to other diagnoses such as phobic/anxiety states and even unipolar depression and, if pursued, would result in only a small number of highly selected families being studied. Another approach is to define affected relatives broadly to avoid making prior assumptions about the status of the different psychoses.

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Biological markers such as auditory P300 and smooth pursuit eye tracking, which are associated with illness in multiply affected schizophrenic pedigrees, may help to resolve the problem of the clinical definition of the schizophrenia spectrum.

The methods of linkage analysis using polymorphic DNA markers has recently led to remarkable progress in our understanding of a growing number of inherited diseases including several that affect the nervous system. These include Huntington's disease, Friedreich's ataxia and Alzheimer's disease, and there is now a realistic prospect of developing novel forms of treatment and prevention for these inherited conditions. We can reasonably expect that similar progress will be made in understanding the inheritance of the major functional psychoses, although linkage results to date have been confusing and sometimes conflicting. A single report has linked schizophrenia with markers in 5q11–13 region (Sherrington et al. 1988), but linkage has been excluded from the same region in other schizophrenic pedigrees (Kennedy et al. 1988; St. Clair et al. 1989; Detera-Wadleigh et al. 1989).

Linkage analysis (Morton 1955), which aims to test the non-independent transmission within families of an illness and a genetic marker, requires several assumptions about the genetics of the disease being investigated. The mode of transmission of the illness must be understood, so that values can be attached to genetic parameters, including the gene frequencies and the penetrances of homozygous and heterozygous states and, above all, it is essential to be able to recognise the phenotype. Diagnostic uncertainty is a particular problem for genetic studies in schizophrenia and the affective psychoses because relatives of schizophrenic probands share an increased risk of schizophrenia and a variety of other psychiatric diagnoses (Kendler et al. 1985). Epidemiological studies by Gershon et al. (1988) have found an increased morbid risk for affective disorders in the relatives of schizophrenic subjects and have failed to identify a clear separation of affective and schizophrenic disorders.

Debate about the Kraepelinian distinction between schizophrenia and manic depressive illness continues (Crow 1986; Kendell 1987), and the issue is illustrated in recently published linkage studies involving families of schizophrenic probands. Sherrington et al. (1988) excluded from their study all families in which cases of bipolar illness were found, but found the strongest evidence for linkage to the chromosome 5q11–13 region when the schizophrenia phenotype was defined to include major and minor depressive illness, phobic disorder, alcoholism and drug use disorder. Kennedy et al. (1988) excluded linkage to 5q11–13 region in a single large Swedish pedigree in which there were very few relatives with affective disorders and there were no cases of schizoid or schizotypal personality disorder. St. Clair et al. (1989) reported 14 Scottish pedigrees, each with two schizophrenic probands. In six of these families there were no cases of bipolar illness amongst the relatives of the schizophrenic probands and in 8 families bipolar illness was diagnosed in at least one relative. Linkage between schizophrenia and 5q11–13 markers was excluded in both the "pure" schizophrenic families and in the families in which bipolar illness was also present. Detera-Wadleigh et al. (1989) excluded linkage to 5q11–13 in five schizophrenic families, two of which had cases of bipolar disorder.

The occurrence of schizophrenia and bipolar illness together in the same large pedigrees described in these linkage studies could be attributed to the segregation of two separate genes – one for schizophrenia and one for bipolar illness (Byerley 1989).

An alternative explanation is that the selection of pedigrees with large, multiply affected sibships suitable for linkage studies in schizophrenia could be biased towards the ascertainment of parents with a relatively good outcome of illness with more "affective" and fewer "schizophrenic" features since prominent and persistent "schizophrenic" symptoms would be expected to be associated with a greater reduction in fertility. This view is supported by an examination of eight "mixed" bipolar/schizophrenic families reported by St. Clair et al. (1989). In five of these families a parent of schizophrenic offspring was diagnosed as bipolar and in the remaining three families the parent who was the obligate carrier had no psychiatric diagnosis (2 families) or minor depression (1 family).

If the selection criteria for "affected" relatives for use in schizophrenia linkage studies is to include a broad spectrum of psychotic and affective disorders as proposed by Gershon et al. (1988), the use of biological trait markers could further clarify the definition of the phenotype.

Biological Markers in Schizophrenia

The criteria a trait marker must satisfy to be of potential use in genetic studies include the presence of the abnormality in a high proportion of cases and its independence from the clinical state at the time of testing. It must be under genetic control and should co-segregate with illness in families of ill probands (Reider and Gershon 1978). Many markers have been proposed in schizophrenia. Neurochemical and pharmacological approaches have tended to focus on the monoamine system (review by Baron 1986) and recently spiperone binding to lymphocytes has been shown to have many of the characteristics of a useful marker (Bondy et al. 1987, 1989).

However, the most promising candidates to date have been physiological measures. Holzman has reported an inefficiency of smooth pursuit eye movements with increased saccadic intrusions and saccadic tracking in over 50% of schizophrenic patients as opposed to only 8% of controls (Holzman et al. 1973, 1977). Eye movement disorder appears stable with respect to neuroleptic medication and clinical state (Iacono et al. 1981; Levy et al. 1983) and twin studies have shown a high level of heritability (Holzman et al. 1978, 1980). In families of schizophrenic probands about 45% of unaffected parents and siblings have eye movement disorder (Holzman et al. 1974, 1984), leading Matthysse et al. (1986) and Holzman et al. (1988) to propose that the clinical symptoms of schizophrenia and disordered eye movement are both expressions of an underlying "latent" trait that is genetically transmitted.

The spontaneous electroencephalogram has been widely reported as abnormal in schizophrenia, but changes are not specific or reliable enough for use as vulnerability markers. Averaged event-related potentials have proved more promising. Many studies have shown a reduced amplitude of middle and long-latency averaged event-related potential responses in schizophrenia following auditory, visual and somatosensory stimuli (Shagass et al. 1977, 1978). In unmedicated schizophrenic patients the P50 component of the auditory-event-related response to paired clicks often fails to habituate, thus distinguishing the response found in schizophrenics from that in normal controls. This may reflect an inability in patients to filter incoming information selectively (Adler et al. 1982; Freedman et al. 1983). In a family study Siegal et al. (1984) reported a loss of P50 habituation in approximately one-half of the siblings and usually one parent of schizophrenic probands.

The so-called P300 response generated when a subject is presented with a signal that is surprising or unexpected has a reduced amplitude and a prolonged latency in schizophrenia that may also be a reflection of disordered selective attention and stimulus evaluation. The latency increase is independent of medication and clinical state (Blackwood et al. 1987) and is found in patients with borderline/schizotypal personality disorder, but not in a group of hospitalised patients with other personality disorders and neuroses (Blackwood et al. 1986; Kutcher et al. 1988). Prolonged P300 latency distinguishes unipolar from bipolar disorders but does not distinguish between bipolar illness and schizophrenia (Muir et al., submitted). Monozygotic twins have very similar P300 responses, which suggests a genetic basis for the generation of the wave form (Polich and Burns 1987).

Both P300 and eye tracking abnormality are present in a higher than expected proportion of first-degree relatives of schizophrenics, including some relatives who have no psychiatric symptoms (Blackwood et al. 1990).

P300 Latency and Eye Movement Disorder a Vulnerability Markers in Schizophrenia Linkage Studies

P300 and eye tracking were measured routinely in the schizophrenic pedigrees recruited for linkage studies described by St. Clair et al. (1989). Because of the effect of age on P300 (Blackwood et al. 1989), these physiological measurements were analysed only in relatives up to the age of 60 years. P300 was recorded as fully described by Blackwood et al. (1987). Briefly, electroencephalographic recordings were obtained from the vertex position referenced to the left ear lobe while the subject performed an "odd ball" task, listening through head phones to a series of low-pitched tones (1000 Hz) interspersed randomly with high-pitched tones (1500 Hz) in a ratio of 9:1. Subjects were asked to attend to the "rare" high pitched tones. In the same subjects eye movements were measured following the method of Holzman et al. (1974), slightly modified as described by Muir et al. (in press).

Table 1 shows that the mean P300 latency was prolonged and eye tracking was disordered in a group of 93 schizophrenic patients compared to non-psychiatric controls. Amongst members of schizophrenic pedigrees P300 latency was considered "abnormal" if it exceeded 346 ms and eye tracking was abnormal if the signal to noise ratio was less than 3.1 (i.e. 2 standard deviations from control means). Table 2 shows the number of subjects in 14 pedigrees with abnormal P300, eye movements or both. Twenty-four of the 39 schizophrenics (62%) showed a physiological abnormality when 5% would be expected by chance. Four out of 11 (36%) relatives with bipolar illness had abnormal P300 and 19 out of 73 (26%) of relatives with no psychiatric diagnosis showed an abnormality.

Table 1. Mean P300 latency and eye tracking

	P300 Latency (ms) Mean ± SD (*n*)	Eye tracking (Signal noise ratio)
Control	301 ± 22.6 (212)	4.95 ± 0.92 (135)
Schizophrenia	338 ± 35.3 (94)	4.24 ± 1.16 (57)

Table 2. RDC Diagnosis P300 latency and eye tracking in 160 family members in 14 pedigrees

Diagnosis	Abnormal P300 (>346 ms)	Abnormal Eye movement (<3.1)	Both P300 and eye movement abnormal	Normal P300 and eye movement
Schizophrenia (schizoaffective, unspecified functional psychosis)	18	2	4	15
Bipolar I and II	4			7
Major depressive disorder (unipolar)	4		1	2
Other diagnosis		3	1	3
No psychiatric diagnosis	10	7	2	54

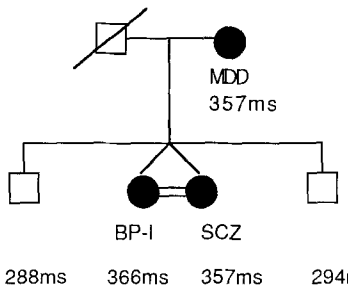

Fig. 1. A family in which monozygotic twins have bipolar (*BPI*) and schizophrenic (*SCZ*) illness and their mother has major depressive disorder (*MDD*). P300 latency (MS) is significantly prolonged in the mother and both twins but normal in the asymptomatic brothers. (A P300 latency of 346 ms is two standard deviations above the control mean)

Figure 1 is an unusual though not unique family (e.g. McGuffin et al. 1982), which serves to illustrate some of the main findings of this study of P300 disorder in relatives of schizophrenic probands. These monozygotic twins and their mother were independently diagnosed by psychiatric colleagues who were unaware of the purpose of the study using the Schedule for Affective Disorder and Schizophrenia (SADS) interview. Zygosity was confirmed by blood grouping. The research diagnoses were in accord with the clinical diagnoses in each case. The manic twin was on treatment with lithium and the schizophrenic co-twin with neuroleptics. P300 latency was significantly prolonged in each of the ill members of the family irrespective of the diagnosis whereas the asymptomatic brothers had completely normal P300 latency. This family provides clinical and physiological support for the view that the occurrence of bipolar illness and schizophrenia in the same pedigree should not invariably be attributed to the segregation of two separate genes in the family.

Linkage Analyses in Schizophrenic Pedigrees

These results are an extension of those reported previously by St. Clair et al. (1989) with the data slightly revised to include three allele changes which resulted from further analyses. A case of non-paternity included in the original analysis has been detected and the subject excluded from this analysis. The incorporation of these changes to the original data has increased the negative lod scores of linkage of schizophrenia to 5q11–13 markers, confirming the original report. The two probes which are most informative were D5S76 (p105–599Ha) and D5S39 (p105–153Ra), which in our study showed linkage to each other (lod score = 3.5) maximally at a recombination fraction of 0.12, the recombination distance reported by Leppert et al. (1987).

The data were analysed using four separate models:

Model A

Cases included: schizophrenia, schizoaffective disorder and unspecified functional psychosis. (Penetrance was 44% calculated from a larger group of 19 schizophrenic pedigrees.)

Model B

As in Model A but also including affective disorders (bipolar and major depressive disorder). Penetrance was 77%.

Model C

As in Model B, restricting the analysis to the six families that had cases of schizophrenia and major depressive disorder only and no cases of bipolar illness.

Model D

P300 latency was used as the sole criterion to define "cases". In the normal and the schizophrenic population P300 latency conforms to a normal distribution, and this variable was entered into the MLINK programme as a continuous variable.

Data were entered into the LINKAGE programmes (Lathrop and Lalouel 1985). The two-point lod scores at specified recombination factions calculated using MLINK programme are listed in Table 3. Close linkage of schizophrenia to the two probes was excluded using all diagnostic models.

The results of three-point analysis across the reported linkage region 5q11–13 are presented in Fig. 2. The (sex average) map locations for the markers were taken from Leppert et al. (1987). In Models A and C, which exclude data from bipolar individuals linkage is excluded (lod score < -2) between about 10 cM from each marker. When P300 latency is entered into the calculations as a continuous variable, linkage is also excluded between these two markers and about 5 cM beyond.

Table 3. 2 point lod scores of linkage of schizophrenia to 5q11–13 markers

Locus	Model	Recombination fraction				
		0.0	0.05	0.10	0.20	0.30
D5S76	A	− 7.6	−3.8	−2.2	−0.7	−0.1
	B	−17.9	−8.9	−5.7	−2.5	−0.9
	C	− 9.8	−4.9	−3.0	−1.2	−0.3
	D	− 3.4	−2.4	−1.7	−0.7	−0.3
D5S39	A	− 5.6	−3.9	−2.8	−1.5	−0.8
	B	− 7.6	−4.6	−3.2	−1.5	−0.6
	C	− 4.9	−3.1	−2.1	−1.0	−0.4
	D	− 2.5	−2.0	−1.4	−0.7	−0.3

Discussion

These results confirm and extend an earlier report that showed no linkage between schizophrenia and the 5q11–13 region in 14 families. The failure to support the findings of Sherrington et al. (1988) is not due to differences in diagnostic procedures or the definition of affected individuals amongst relatives. The same diagnostic interview was used (Schedule for Affective Disorders and Schizophrenia, Life Time version) and Research Diagnostic Criteria and DSM III R were adhered to. Differences cannot be attributed to the inclusion in the Scottish study of some relatives with bipolar illness because linkage to the 5q11–13 region was excluded in six families which had no cases of bipolar disease (Model C) and also by the analysis using only cases of schizophrenia, schizoaffective disorder and unspecified functional psychoses (Model A).

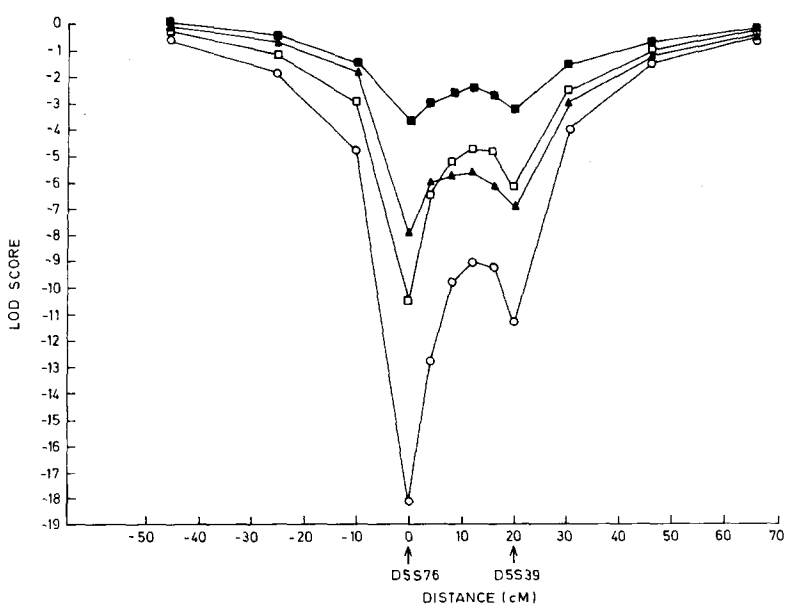

Fig. 2. Three point lod scores comparing the location of a possible schizophrenia locus relative to markers D5S76 and D5S39 completed using the LINKMAP programme of LINKAGE (Lathrop and Lalouel 1985). Models A to D define the phenotype in various ways as described in the text. ▲ Model A; ○ Model B; □ Model C; ■ Model D

Bipolar illness was diagnosed among relatives in 8 out of 14 families in this study and in 2 out of 5 families by Detera-Wadleigh et al. (1989). This is unexpectedly high since most family studies show a very small or no increase in bipolar illness in schizophrenic families. However, for linkage studies, families are selected because they are multiply affected and have large sibship size, and this may bias towards the selection of families in which parents of affected children have symptoms which are more "affective" than "schizophrenic". There is little justification for excluding these families entirely from linkage studies, as it is premature to conclude that two separate genes are segregating to cause these different psychoses, and in some families (Fig. 1) this is clearly not the case.

Gershon et al. (1988) proposed that in linkage studies the definition of affected relatives should include a broad spectrum of psychotic and affective disorders, and this view is supported by the distribution of physiological markers in schizophrenic families. Prolonged P300 latency is found at the population level in schizophrenia, bipolar illness and in borderline/schizotypal personality disorder. Amongst relatives of schizophrenic probands P300 abnormality is found in subjects with a range of psychiatric diagnoses and in a some relatives who are asymptomatic. The interpretation of the physiological data is speculative and will be convincing only if positive linkage is found. However, combined with brain imaging studies and psychological assessment, physiological abnormalities in relatives of schizophrenic probands may help to define the spectrum of schizophrenia and provide a reliable basis for defining affected relatives.

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