Studies of blood groups, genetic linkage, trait association, and chromosomal pattern in multiple sclerosis

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Occasional mention of the familial occurrence of multiple sclerosis in the early literature of the disease (Eichhorst, 1896; Reynolds, 1904; Curschmann, 1920) arose from its recognition in isolated cases. However, conclusions as to the possible importance of this finding in the aetiology of the condition varied widely. During the last two decades interest has been stimulated in familial multiple sclerosis as a result of more extensive and detailed studies, notably those of Curtius (1933), Curtius and Speer (1937), Mackay (1950), Pratt, Compston, and McAlpine (1951), and Müller (1953). A significantly increased incidence of multiple sclerosis in near relatives of patients suffering from the disease was established by these and subsequent studies. McAlpine (1946) reported eight examples in a series of 142 patients with multiple sclerosis and considered this prevalence to be significant, possibly implying an inherited constitutional factor which might render an individual more susceptible to the disease. Pratt and his colleagues (1951), from a study of 310 patients, also postulate the presence of an inherited disposition to the disease which might potentiate a second aetiological factor.

Allison and Millar (1954) and Sutherland (1956) also found increased familial prevalence in their surveys of Northern Ireland and Scotland. Myrianthopoulos and Mackay (1960) studied multiple sclerosis in 54 pairs of twins and their relatives. The twin study did not permit inferences as to whether the disease was more frequent among monozygotic as opposed to dizygotic twins. They felt that the results of the investigation among relatives, however, were compatible with the hypothesis that multiple sclerosis is determined by a pair of autosomal recessive genes with about 43% penetrance.

In view of a widespread implication that the evidence favoured a major genetic contribution to the aetiology of multiple sclerosis further investigations were based on a group of families in which more than

one member was affected by the disease which came to our notice during the course of a survey of multiple sclerosis recently carried out in the counties of Northumberland and Durham (Poskanzer, Schapira, and Miller, 1963).

In the general population of Northumberland and Durham the prevalence rate of multiple sclerosis is 5.0 per 10,000 or 0.050%. The prevalence rate of siblings of patients with multiple sclerosis is 115.0 per 10,000. The expectation that an offspring of an affected parent will develop the disease is 58.0 per 10,000. Rates for offspring and siblings together (98.5 per 10,000) represent a 7.8-fold increase in prevalence in families when compared with the rate in the general population, though the risk to a second member of the family is admittedly only 0.58%, or one chance in 172 (Schapira, Poskanzer, and Miller, 1963). Analysis of our family data revealed that when multiple cases occur in a family they occur twice as often in sibs as in parent and child which would suggest a recessive mode of inheritance.

Attempts were therefore made to establish evidence either of genetic linkage by the sib-pair method of Penrose and the sequential method of Morton, or to establish an association between the common inherited blood groups and the occurrence of the disease.

A search for linkage or association attempts to establish that two separately recognizable hereditary qualities occur together in families. The situation is well recognized, for example, in the occurrence of certain hereditary diseases linked with sex. Genes situated on the same chromosome pair have a relationship to one another which is termed genetic linkage. In the process of maturation 'crossing over' can occur between a pair of chromosomes. During meiosis, the cell division in which the number of chromosomes is reduced from 23 pairs to 23 single

chromosomes, there is an interchange of homologous portions between the two paired chromosomes which results in a rearrangement of gene pairs on the two chromosomes. If two genetic loci are close together, they are unlikely to be separated. The result is to produce a set of inherited characteristics which result from genes which are closely linked. If genes for two traits are close together the linkage effect will be marked. If they are far apart the effect will be difficult to observe. In addition the two loci on the same chromosome may be so far apart that they assort freely and appear unlinked. Extensive studies for linkage have been carried out in experimental animals but the small size of families and randomness of human matings present complex statistical and mathematical problems partially solved by two methods employed here.

METHODS

The present study was based on 700 consecutive cases of multiple sclerosis reported by medical practitioners to a multiple sclerosis unit in the Neurological Department at Newcastle. Of these 65 were rejected because the diagnosis was considered incorrect or equivocal or because the patients lived outside the counties of Northumberland and Durham and may have been attracted to Newcastle because of our special interest in the disease. Information was sought on the parents and siblings of the remaining 635 patients.

Twenty cases were excluded from the tabulation of siblings and 24 from the parent tabulations because information about siblings or parents of *propositi* was inadequate. In eight of the families studied, involving 16 *propositi*, two siblings were both *propositi*, reducing the total number of families studied for siblings to 607 (635, -20, -8) as against 603 (635, -24, -8) for parents and children.

There were 24 families with two or more members in whom a diagnosis of multiple sclerosis was established with reasonable certainty. In 13 families both affected individuals were personally examined, whilst in the remainder one case was examined personally and the second was considered to be reliably established on the basis of information obtained from another neurologist or from post-mortem findings. In 17 instances both cases occurred in siblings, in seven instances in parent and child. A second unequivocal case of multiple sclerosis therefore occurred in 4.0% of families investigated (24/607).

In six further families there was a second member in whom the diagnosis of multiple sclerosis was 'possible', but the data available were considered

inadequate to establish it to our satisfaction. If these six patients were in fact all suffering from multiple sclerosis the percentage of families with more than one person affected would be 4.9 (30/607). When five established cases found in collateral blood relations outside the immediate family are included, *i.e.*, grandparents, first cousins, uncles, and aunts, to make the basis of assessment comparable with that used in previous similar studies, the rate becomes 5.8%. The data relating to familial investigations have been published elsewhere (Schapira *et al.*, 1963). Further intensive study was undertaken of eight families in which more than one affected member was living. The total number of individuals in the eight families studied was 51 (34 offspring and 16 parents). Of this group, 16 had multiple sclerosis and two retrobulbar neuritis (17 offspring, one parent). Blood groups were studied in 34 of the 35 siblings, and in six of the parents. One sibling with multiple sclerosis died before the investigation was completed; the parents not studied were no longer living. Partial information about blood groups of the 10 parents who were no longer living could be deduced from some characteristics of the blood groups of their offspring.

In addition to the eight families, each of which included two or three members with established multiple sclerosis and from whom blood was drawn because they were undergoing intensive study for other purposes, six additional individual patients were evaluated. With the addition of these six non-familial cases of multiple sclerosis, the total number of individuals investigated for blood grouping was 46, of whom 23 had multiple sclerosis or retrobulbar neuritis.

At the present time it is possible to study 12 common known genes each presumed to be situated on one of the 23 pairs of chromosomes, and to try and establish linkage of a trait presumed to be inherited with one of these genes. The traits studied included the nine common blood group systems (ABO, Rh, MNS, P, Kell, Lewis, Kidd, Duffy, and Lutheran). In addition, the genetically determined ability to taste phenylthiocarbamide (P.T.C.) was tested, using a 50 mg. per litre screening solution (one part in 20,000). The sex chromosomes comprise an additional chromosome pair. Secretion of ABH and Lewis substances in the saliva were also investigated.

In one instance blood group analysis revealed that the firstborn of seven siblings could not have been the child of the stated parents. The offspring's MNS blood grouping was MNss which would require the parents' blood groups to include at least one group N. However, the parents' blood groups derived from the MNSs pattern of the other six

children must in fact have been MSMs and MSMs. The blood of the child in question was re-examined after the inconsistency had been discovered and the original findings were confirmed. Because of this discrepancy it was necessary to omit this individual from subsequent tabulations of linkage for all blood groups.

GENETIC LINKAGE

The results of linkage analysis by the sib-pair method of Penrose (1953) are summarized in Table I. This method is of particular value when the parental genotypes are unknown, though in fact only a small fraction of the available genetic information is utilized. When one of the test characters is a rare recessive trait, however, the sib-pair method makes better use of available data. The method aims to eliminate the necessity for obtaining data from two generations and attempts to minimize the possible complicating factor of age variation in the manifestation of the traits under study.

TABLE I

LINKAGE SCORES FOR DISSEMINATED SCLEROSIS WITH VARIOUS MARKERS ESTIMATED BY THE PENROSE (1953) SIB-PAIR METHOD

Marker	No. of Pairs (n)	ηSC (1)	$\eta^2 SI$ (2)	$SC/\sqrt{SI}$ (3)
Sex	78	-2.81	57.08	-0.37
ABO	73	-8.87	97.17	-0.90
Rhesus	73	-8.72	40.50	-1.37
MN	73	-3.93	8.39	-1.36
Ss	73	-3.23	169.15	-0.25
P		(uninformative)		
Kell	73	-6.34	41.05	-0.99
Lewis	73	-1.69	20.64	-0.37
Kidd	73	-5.11	54.04	-0.70
Duffy	73	-2.12	47.26	-0.31
Lutheran	73	-0.35	25.97	-0.07
ABH secretor	65	-1.61	15.77	-0.41
Taste P.T.C.	65	-5.47	47.89	-0.79

(1) Score for estimating linkage

(2) 'Information'

(3) Linkage estimate divided by its standard error.

The sib-pair method can also be applied to traits in which the mode of inheritance is unknown. In this instance it failed to reveal any evidence of linkage. In no instance was the estimate of linkage greater than twice its own standard error (Table I) which would have suggested a significant linkage. The number of families available for study was small. The data will be provided for the use of other investigators who may be able to examine families additional to those presented here.

Morton (1955 and 1957) has adapted the method of sequential analysis to the study of linkage. This method makes use of the fact that information on possible linkage is accumulated as a succession of

samples each of which is quite small relative to the amount of data required to detect even moderately close linkage. It consists in determining, as data are collected, whether enough information is available to decide that there is no linkage, that there is linkage of a specified degree, or that such a decision cannot be made one way or the other. Employing his method the total z score for each family is given in Table II.

TABLE II

LINKAGE SCORES FOR DISSEMINATED SCLEROSIS WITH VARIOUS MARKERS ESTIMATED BY THE MORTON (1955, 1957) SEQUENTIAL METHOD

Marker	Z-Scores Total for All Families
ABO	-3.7829 ¹
Rhesus	-2.8384 ¹
MNS	-2.5601 ¹
P	0.1792
Kell	-1.8732
Lewis	-0.2232
Kidd	-1.8329
Duffy	-0.7370
Lutheran	-1.5659
ABH secretor	-2.2368 ¹
Taste P.T.C.	-2.7670 ¹

theta = 0.05.

¹Recombination fraction significantly greater than theta. Close linkage unlikely.

If the cumulative z score is greater than 3 there is significant evidence for linkage. If the cumulative z score is between -2 and 3, evidence for linkage is not decisive and additional data should be collected. If the cumulative z score is less than -2 then the recombination fraction is significantly greater than theta (in this case 0.05) and the possibility of close linkage can be rejected with some confidence. Employing Morton's method, z scores less than -2 were found in five instances. The remaining scores fell between -2 and 3, indicating that further information is required. A score in excess of 3 furnishing evidence of linkage was not obtained. Because of the small amount of data available, attempts were not made to recalculate for values of theta greater than 0.05, which might reveal less close linkage. In a number of instances the tables provided by Morton did not extend to the family types required and scores were calculated from his original formulae.

TRAIT ASSOCIATION

In the absence of linkage an attempt was made to determine if a simple association between multiple sclerosis and any of the inherited characteristics was present. The percentages of patients positive for each inherited characteristic are summarized in Table III and compared with the expected rate in the general population as given by Race and Sanger

OCCURRENCE OF CERTAIN INHERITED CHARACTERISTICS IN 23 CASES OF DISSEMINATED SCLEROSIS COMPARED WITH THE GENERAL POPULATION

The detailed results of the tests of various blood groups and other inherited characteristics for both affected and unaffected subjects have been omitted

One 54-year-old male patient with definitely established multiple sclerosis was studied by Dr. John E. Gray for chromosomal abnormality. The karyotype was normal 46/XY. The chromosome counts in 12 cells showed 46 chromosomes; three other counts of 43, 44, and 48 chromosomes were also determined. Some cells showed satellites on a number 22 chromosome as well as on the 21st and 15th chromosomes, but these variations were regarded as of no significance.

The recognition that multiple cases of disseminated sclerosis occur in about 6% of families has led many authors to suggest that a genetic factor is important in aetiology. In our previously reported series multiple cases occurred twice as often in sibs as in parent-child relationships, suggesting that any hereditary factor involved was likely to be of a re-

but they will be provided by the authors on request. Additional cases may be added to these data in order to make available an increasing amount of information for analysis.

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