Genetic studies of acute infantile spinal muscular atrophy (SMA type I)

An analysis of sex ratios, segregation ratios, and sex influence

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SUMMARY An analysis of segregation and sex ratios, and of sex influence, was undertaken in a series of 78 index patients with acute infantile spinal muscular atrophy (SMA type I). The sex ratio of index patients was 2.0, and the excess of males was shown to occur principally among sporadic cases. The sex ratio of familial cases did not differ significantly from 1.0. The implications of this are discussed. No sex influence on age at onset, or on life expectancy, was present. The segregation ratio (Weinberg Proband method) was 0.29 for all index cases, and 0.26 for all cases excluding those referred specifically to a genetic counselling clinic. Autosomal recessivity is confirmed for this disease, with the probable inclusion of unrecognised male phenocopies in clinical series.

Acute fatal infantile spinal muscular atrophy (SMA type I) is due to a common gene, with a carrier frequency of 1 in 80 in the English population (Pearn, 1973a). In spite of its relative importance as a cause of childhood mortality in that group of diseases due to genetic factors (Roberts *et al.*, 1970), no formal study of segregation or sex ratios has been undertaken on a series of index cases defined clinically as SMA type I.

Three major studies have undertaken a segregation analysis of SMA that has included many patients with this specific disease (Brandt, 1950a; Winsor *et al.*, 1971; Emery *et al.*, 1976). However, all have included index patients with the chronic form or forms of this disease (types II and III SMA of Emery, 1971; chronic proximal SMA of Bunday and Lovelace, 1975). Certain questions of sex incidence also remain unresolved. Both Smith and Patel (1965) and Pearn (1974) found an unexpected predominance of males with this condition. Emery *et al.* (1976) also found an excess of males in the International Collaborative Study, but not an excess of sibships with only males affected. Brandt (1950b) and Winsor *et al.* (1971) found males and females equally affected; these latter 2 studies were the only 2 to test observed differences from an expected ratio of 1.00, using formal techniques.

For these reasons, it seemed appropriate to look in depth at the genetic implications of this disease, to confirm autosomal recessivity as the mode of inheritance involved, and to resolve the uncertainty about sex incidence.

Methods

CLINICAL SERIES

The study was undertaken on a combined consecutive unselected series of patients with acute fatal SMA (type I), as clinically defined by Pearn *et al.* (1973) and Pearn and Wilson (1973). There were 78 index patients from 72 families and 24 secondary cases. A detailed account of each family is given in Appendix 2 (first 27 families) and Appendix 6 of Pearn (1974). The series comprised every case of this disease presenting to, or traced from, the Hospital for Sick Children, Great Ormond Street, London, and the Regional Neurological Centre, Newcastle-upon-Tyne, over the 10-year period 1961 to 1970, inclusive. An index patient was defined as a child with the disease independently ascertained, who brought the family to the notice of the study. Of the families, 7 were not interviewed personally for this study, but reliable information was obtained. In the remainder (65 families), at least one parent was reinterviewed in

every case, living sibs were examined, and details of deceased index patients and affected sibs obtained. Full hospital records were obtained and examined for each of the 72 index patients, and for many of the secondary cases as well. The series of index patients traced from the Hospital for Sick Children, London, included 7 from the Genetic Clinic, referred from clinical sources outside the hospital. This latter type of case requires special consideration in the segregation analysis.

SEX RATIOS

The sex ratio was defined as the proportion of affected male index patients to female index patients. In this study the observed sex ratio was tested using the null hypothesis that the observed proportion did not differ from the 1.00 expected if the gene concerned was autosomal recessive. The standard appropriate test (Armitage, 1971) was used, with and without continuity correction. It has been shown that this disease occurs in the first few months of life, with death having occurred by 7 months of age in 50% (Pearn and Wilson, 1973). At least one-third of affected infants manifest prenatal signs of the disease (Pearn, 1973b). Under these circumstances, the theoretical expected sex ratio of 1.00 is not appropriate in view of the normal male predominance in live births. For this reason, the observed sex ratio of the SMA series was tested against expected values of both 1.00 and 1.059. The figure of 1.059 was obtained from the Registrar General's Fertility Analysis tables (1972), and was the mean sex ratio of live births for the 11-year period from 1960 to 1970, inclusive. A sex ratio of 1.059 corresponds to a male-to-total population ratio of 0.514. Sex ratios were tested separately in familial and in non-familial (that is, sporadic) index patients, in addition to the total series.

SEX INFLUENCE ON CLINICAL SEVERITY

Two quantifiable features of the clinical aspects of the disease were available for this analysis. These were age at clinical onset and age at death. It was not possible to specify the average time when the disease begins in that group manifesting prenatal onset (Hausmanowa-Petrusewicz *et al.*, 1975); an arbitrary score of -1 month was ascribed to index patients who

showed clinical evidence of the disease at birth. Means, variances, and standard errors were calculated for each sex separately. When the variance ratio (F) was not significant at the 1% level, a 't' test was used. If the variances of the quantified disease manifestations did differ significantly, a Welch's test was used and the result read against tables (Pearson and Hartley, 1958).

SEGREGATION ANALYSIS

Identification of index cases was by multiple incomplete ascertainment and the Weinberg Proband method was used. In addition, the proportion of affected sibs born before and after index patients were calculated separately. This was done as an additional study to obtain an indirect quantitative measure of any limiting of family size that might occur after the birth of an affected child.

Results

SEX RATIOS

Sex ratios are shown in Table 1. It can be seen that in this total series of 102 patients there was a significant excess of male infants. The sex ratio of all index patients was 2.0. If the male incidence was expressed as a proportion of the total number of index patients, the proportion was 0.67 (SE 0.05) with a 95% confidence range of 0.57 to 0.77. This was significantly different from the predicted proportions of both 0.500 and 0.514 ($P < 0.01$ in both cases). It can be seen from Table 1 that the excess of males occurred in the non-familial (isolated) patients who were necessarily all index cases and who, as a group, had a sex ratio of 2.75 (male proportion of 0.73, SE 0.07). The sex ratio of familial index patients was 1.28 (proportion of males affected was 0.56). This did not differ significantly from the sex ratios corresponding to male proportions of either 0.500 or 0.514.

SEX INFLUENCE ON CLINICAL SEVERITY

A comparison of clinical features of the disease in the 2 sexes is shown in Tables 2 and 3. Considering only index patients, the variances for age at death were significantly different between the 2 sexes ($F = 2.20$; $P < 0.01$). A Welch's test (Pearson and Hartley, 1958) showed, however, that the means were not

Table 1 Sex ratios in SMA type I

	Total patients	No. of males	Sex ratio (M:F)	Proportion of males affected (M:M + F)	Standard error	Significance of difference (P) of male proportion from	
						0.500	0.514
All index patients	78	52	2.00	0.67	0.05	$P < 0.01$	$P < 0.01$
All patients (index plus secondary patients)	102	65	1.76	0.64	0.05	$P < 0.01$	$0.01 < P < 0.02$
Familial patients only	57	32	1.28	0.56	0.07	$P < 0.35$	$P < 0.40$
Non-familial (sporadic) patients only	45	33	2.75	0.73	0.07	$P < 0.01$	$P < 0.01$

Table 2 Sex influence on clinical severity. Data from 78 index patients (52 males and 26 females) with SMA type I

	Age at death (m)			Age at clinical onset (m)		
	Mean	Standard error	Range	Mean	Standard error	Range
Males	7.63	0.54	1.5-18.5	0.57	0.20	Prenatal-4
Females (all index patients)	9.17	1.13	1.0-30.0	0.73	0.26	Prenatal-4.5

Table 3 Sex influence on clinical severity. Formal testing of age at death and age at onset

Clinical feature	Comparison	Variance of ratio (F)	Significance of (F)	Difference between means	
Age at death	Males v. females	2.20	P < 0.01	d = 1.232 (Welch's test)	P > 0.10
Age at clinical onset	Males v. females	1.211	P > 0.05	t = 0.475 (76 DF)	P > 0.5

Table 4 Segregation analysis for SMA type I: 78 index patients (71 index patients excluding Genetic Clinic families)

	Sibs born before index patient		Sibs born after index patient		Segregation ratio	Standard error
	Normal	SMA type I	Normal	SMA type I		
Familial index patients only	25	23	8	12	—	—
Sporadic index patients only	28	0	22	0	—	—
All index patients	53	23	30	12	0.29 (35/118)	0.042
All index patients except Genetic Clinic families	48	17	24	9	0.26 (26/98)	0.045

significantly different. Considering age at clinical onset, the variances for each sex did not differ significantly, and a 't' test revealed no difference between the means for each sex (Table 3).

SEGREGATION RATIOS

The segregation analysis is shown in Table 4. The percentage of affected sibs of index patients was 29.6%, with a 95% confidence range of 21.4 to 37.8%. Families referred specifically to a genetic clinic tend to bias this ratio (C. O. Carter, 1978, personal communication), as it is often impossible to decide who are true index patients, independently ascertained. For this reason, calculations were also made on all patients, excluding those from families traced exclusively through the Genetic Clinic (Table 4). The segregation ratio of this residual group (that is, excluding Genetic Clinic families) was 0.26 with a 95% confidence range of 0.18 to 0.35. This did not differ significantly from 0.25. For the total group, the segregation ratio of sibs born before index patients was 0.30, and that for sibs born after index patients was 0.29, a difference which was not significant ($P > 0.20$). This is direct proof that no limitation of family size occurs in this disease, at least before genetic counselling is sought.

Discussion

The segregation analysis not only confirms autosomal recessivity for this disease, but also that the familial clustering observed in earlier studies (Pearn,

1973a) has a genetic, as opposed to an environmental cause.

The very high sex ratio of non-familial index cases (2.75) is surprising and confirms the trends observed by Smith and Patel (1965) and Emery *et al.* (1976). Of all possible explanations, several warrant special consideration. The first possibility is that there are X-linked recessive cases within this clinical syndrome. This is a worrying possibility as children of a maternal carrier of such a gene will also have, on average, a segregation ratio of 0.25, and new mutations would explain the excess of sporadic male births. If this were so, then one would expect to encounter affected brothers of the mothers of some of the male index cases, but no such case has been observed. For a lethal X-linked recessive gene, one third of sporadic cases are the result of new mutations, and it may be that numbers are too small to overcome chance effects. In addition, as death usually occurs in the first few months of life, mothers carrying such a hypothetical gene may not be aware of, or may have forgotten, a brother who died at the age of (say) 4 months, 40 years before.

The appropriate test of this hypothesis is to consider separately the sex ratio of affected sibs of male index patients and of female index patients. The sex ratio for affected sibs of male index patients was 0.54, which not only was not significantly different from 1.0 ($P > 0.1$), but was a trend in the wrong direction, if X-linked recessive inheritance was present. This test was not so powerful with the current data, however, as it was already established that the unexplained excess

of males occurred only in the non-familial group. The possibility thus remains of a small (unknown) proportion of X-linked recessive cases, but positive evidence to confirm this is lacking.

Another possible theory to explain the observed high proportion of males is to consider selective intra-uterine embryoletality affecting females with the disease. There is no evidence for this from a study of the individual families, and it would be surprising in view of the neutral effects of sex on the postnatal clinical features of the disease, noted also in this study. The question of selection artefact must also be considered. It is possible, though untestable, that because of the social awareness of X-linked muscular dystrophy in the differential diagnosis of a hypotonic infant, male infants might be more readily diagnosed than females with the condition, the latter having a greater tendency to die from 'pneumonia', and so escape ascertainment in such a study as this.

The final possibility is that there is a small proportion of phenocopies among sporadic cases, with an unrecognised sex predilection for males. This seems to be the most likely explanation at this stage of knowledge. Whatever is the true explanation, both theoretical and empirical risks for genetic counselling should follow an assumption of autosomal recessivity for index cases.

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