Absence of genetic heterogeneity in Duchenne muscular dystrophy shown by a linkage study using two cloned DNA sequences

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SUMMARY A linkage study using two different restriction fragment length polymorphisms (RFLPs) identified with cloned DNA sequences has failed to provide evidence for genetic heterogeneity in Duchenne muscular dystrophy (DMD) when tested against intelligence quotient (IQ). Analysis of data for age of confinement to a wheelchair against IQ gave no evidence for heterogeneity. These results are of a practical as well as theoretical significance, since the existence of multiple loci causing DMD would make it more difficult to apply linkage data to genotype prediction in this disease.

Duchenne muscular dystrophy, the commonest and most severe of the muscular dystrophies, is inherited as an X linked recessive disorder. The condition is usually diagnosed before 6 years of age, with presenting symptoms of muscle weakness and abnormal gait. Confinement to a wheelchair normally occurs between 8 and 12 years of age, and death often occurs in the late teens or early 20s.^{1 2}

Early psychomotor delay is a frequent feature in affected males, and around 30% of patients have significant mental handicap with an IQ of 70 or less. This has been shown not to be a direct consequence of the physical handicap produced by the disease, and a strong correlation for mental handicap has been noted within families.³

These findings have led to the suggestion that there may be genetic heterogeneity for DMD, owing either to multiple alleles at the same locus or to separate loci. Emery *et al*⁴ have suggested that such heterogeneity can be shown clinically, those boys showing significant mental handicap having a milder physical course with a later age of confinement to a wheelchair. The apparent high mutation rate in DMD has also been used as an argument in favour of mutation at more than one locus.⁵

Genetic linkage studies can be a powerful tool for

detecting genetic heterogeneity involving separate genetically distinct loci, though they are unable to exclude the existence of closely linked loci or of multiple alleles causing a disease. To date, this approach has not been used to look for heterogeneity in DMD because of a lack of linked marker loci.

In this study, we have combined linkage data for two restriction fragment length polymorphisms (RFLPs), detected with random X chromosome derived cloned DNA sequences, with clinical observations to test the suggestion that there may be two genetically distinct Duchenne muscular dystrophy loci, one associated with significant mental handicap and late confinement to a wheelchair and the other with normal intelligence and relatively early confinement to a wheelchair.

Methods

The diagnosis of Duchenne muscular dystrophy was established by us on the basis of clinical examination, a greatly raised creatine kinase (CK) level, and in the majority of cases either electromyography or muscle biopsy. Information on intellectual state was available on 53 affected males from 32 kindreds, the study being restricted to those currently living, dead sibs, and relations from the same generation. Information on past generations was not included, since IQ assessments were generally not available

and criteria for confinement to a wheelchair not comparable.

The patients were divided into two groups, educationally subnormal (those with IQ of 70 or below, or who were unable to benefit from normal education) and others. Either a formal IQ or record of school progress was obtained on all but three patients. Two of these were grossly subnormal when seen in their homes, and the third, who had successfully completed two years of a university course, was included with the normal IQ group. In three kindreds analysed for genetic linkage, educationally subnormal (ESN) patients and those with a normal IQ occurred together. These were classified as ESN for purposes of genetic linkage analysis, as in each case more than half of the affected subjects in the family were in the ESN category. A fourth family with two boys, one of whom was ESN and the other of normal intelligence, was not segregating for either probe.

Genetic linkage data for those kindreds informative for either or both of the RFLPs shown to be linked to DMD^{6 7} were analysed by the computer program LIPED; the lod scores for ESN and normal IQ families were calculated separately for two RFLPs. Only obligatory, not possible, carriers were included in the analysis. The methods for typing these polymorphisms, λ RC8 and L1.28, which both show Mendelian X linked codominant inheritance with two alleles, have been reported previously.^{6 7}

Results

Twenty-one kindreds, including four where none of the living affected boys was yet wheelchair-bound, gave linkage information. Table 1 shows the lod scores obtained with the two DNA probes λ RC8 and L1.28, each analysed separately for linkage in informative kindreds (six of whom were segregating

TABLE 2a *Present study: age in wheelchair.*

	No	Mean (year)	SD	t test	p
Normal	29	9.897	1.655	1.133	NS
ESN	18	9.361	1.433		

TABLE 2b *Data of Worden and Vignos⁸: age at first walking.*

	No	Mean (year)	SD	t test	p
Normal	28	3.554	2.092	2.806	<0.01
ESN	10	2.091	1.074		

for both probes) classified as ESN or of normal intelligence. When the linkage data are subdivided according to IQ, the evidence for linkage within the individual groups is weakened, but each category shows positive and comparable scores. The retention of positive linkage for all groups demonstrates that, within the limits imposed by the number of informative families, there is no evidence for heterogeneity of DMD with IQ.

Full details on age at confinement to a wheelchair and school performance were available on 47 children from 28 kindreds. The mean age at confinement to a wheelchair in the two categories is shown in table 2a. The age is increased in the normal group, but does not reach statistical significance. Analysis of the earlier data of Worden and Vignos⁸ in the same way shows a positive correlation between age at first symptoms and IQ, significant at the 1% level (table 2b).

Discussion

The positive and comparable lod scores obtained for both probes with DMD separately for ESN and intellectually normal groups of affected boys

TABLE 1 *Linkage data for Duchenne muscular dystrophy, λ RC8 and L1.28, subdividing according to IQ.*

θ	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	95% confidence limit
λ RC8-normal (n=6)	-1.385	-0.161	+0.218	+0.348	+0.377	+0.353	+0.298	+0.230	+0.154	+0.077	4-50 cM
λ RC8-ESN (n=4)	+0.095	+0.929	+1.215	+1.267	+1.201	+1.061	+0.872	+0.656	+0.430	+0.213	2-39 cM
λ RC8-DMD (n=10)	-1.290	+0.768	+1.433	+1.615	+1.578	+1.414	+1.170	+0.886	+0.584	+0.290	5-37 cM
L1.28-normal (n=10)	+1.743	+2.740	+2.831	+2.664	+2.394	+2.065	+1.696	+1.303	+0.886	+0.451	2-26 cM
L1.28-ESN (n=7)	-4.104	-1.487	-0.527	-0.081	+0.151	+0.259	+0.289	+0.268	+0.208	+0.117	9-50 cM
L1.28-DMD (n=17)	-2.361	+1.253	+2.304	+2.583	+2.545	+2.324	+1.985	+1.571	+1.094	+0.568	7-33 cM

n=number of informative kindreds.

θ =recombination fraction.

strongly indicates that Duchenne muscular dystrophy results from mutations occurring at a single genetic locus on the short arm of the X chromosome (table 1). It cannot, however, exclude heterogeneity resulting from different mutations at the same or closely linked loci.

The absence of any clear cut dividing line in our data between confinement to a wheelchair and increasing IQ argues against a DMD mutation with mental retardation at a genetically distinct locus (table 2a). The reanalysis of the data of Worden and Vignos⁸ (table 2b) also argues against a subgroup of less severely affected DMD boys of low IQ. However, these data must be treated with caution as mental retardation may itself be the presenting symptom leading to a positive diagnosis of DMD.

These findings are not in agreement with the suggestion that patients with early disability have higher IQ levels.⁴ Our study, like others,³ finds a correlation for many but not all kindreds for the occurrence of mental handicap. However, the fact that four families have both ESN and intellectually normal affected boys in itself argues against heterogeneity.

Our evidence favouring a single locus in DMD is of practical importance when linked polymorphisms are used in genotype prediction.⁹ Final resolution of the question of heterogeneity will only come when the molecular defect has been directly identified but at present, despite its high mutation rate, it can be concluded that DMD is probably determined by a single genetic locus on the X chromosome.

This work was supported by grants from the UK

Muscular Dystrophy Group, the Cystic Fibrosis Research Trust, and the US Muscular Dystrophy Association.

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