The application of linkage analysis to genetic counselling in families with Duchenne or Becker muscular dystrophy

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SUMMARY A total of 278 families of probands with Duchenne or Becker muscular dystrophy has been ascertained and offered genetic counselling. Linkage studies have been performed in these families using polymorphic DNA markers identifying loci linked to Duchenne and Becker muscular dystrophy. The clinical features of the probands are discussed: there was marked intrafamilial resemblance in the severity of the disease.

We estimate that a complete study of potential carriers in these families would require analysis of samples from approximately 1400 subjects. The results of linkage studies tended to move women's carrier risk estimates (based on CK and pedigree data) towards the extremes of the risk categories, providing a more definitive risk estimate for 81% of the women who were previously in the middle range of carrier risk probabilities. About 70% of the families had only one affected member. Linkage analysis altered carrier risk estimates in 95% of sisters and aunts of index cases, but only affected estimates of the mother's carrier risks in about 11% of isolated cases. Even where linkage studies were not helpful in elucidating carrier risks, information could usually be obtained for use in prenatal diagnosis if required.

We have assessed the attitudes to pregnancy and prenatal diagnosis of women at risk of being carriers of Duchenne or Becker muscular dystrophy and report 17 pregnancies in these women.

Duchenne muscular dystrophy (DMD) is the commonest and most severe type of dystrophy, inherited as an X linked recessive trait, with an incidence of about 1 in 3000 to 3500 males.¹ Becker muscular dystrophy (BMD) has a similar clinical pattern, but is milder than DMD and between six and 10 times less frequent. About half the males affected with DMD have delayed motor milestones. Pelvic girdle weakness develops gradually, with loss of ambulation by about 11 years of age. Pectoral girdle and distal arm and leg weakness develop progressively and survival much beyond the age of 20 years is unusual. Calf hypertrophy is common, but associated dysmorphic features are not found. Mild mental retardation may occur and ECG abnormalities are common. Creatine kinase (CK) levels are

greatly raised in DMD. The EMG is myopathic and muscle biopsy shows characteristic histology. There is no clinical or laboratory finding which distinguishes between DMD and BMD, apart from the milder nature and slower course of BMD. An arbitrary definition is that boys with BMD retain the ability to walk beyond the age of 16 years.^{2,3}

Because there is as yet no treatment for these disorders, genetic counselling is of particular importance for females at risk of being carriers. They can then make informed decisions about childbearing and whether to avail themselves of prenatal diagnosis.

Until recently, carrier detection in female relatives of boys with DMD or BMD has relied entirely upon pedigree analysis⁴ and estimations of serum CK, with or without EMG and muscle biopsy. These tests can all yield false negative results; for instance one third of obligate carriers of DMD have serum

CK levels within the normal range.⁵ The discovery of polymorphic loci linked to *DMD* and *BMD* offers an additional means of defining carrier risks within families and is also, in principle, applicable to prenatal diagnosis.⁶ Fetal sex can now be determined at chorion biopsy in the first trimester of pregnancy and DNA analysis may be performed on the chorionic villus samples (CVS) obtained by this technique.

To explore the application of linkage techniques to genetic counselling, we approached the families of patients with X linked muscular dystrophies currently attending the Hammersmith Hospital Muscle Clinic and the South-East Thames Regional Genetics Centre, Guy's Hospital. We hoped that previous education, by counselling of a large body of families at risk, would lead to a more rational approach to the clinical use of linked markers for prenatal diagnosis and carrier risk estimates. The uptake of genetic advice, the problems encountered in setting up this service, and the effects of counselling on women's attitudes towards pregnancy will be discussed.

Methods

POPULATION STUDIED

In the initial part of the study, we ascertained all the *DMD* and *BMD* families who had attended the Hammersmith Hospital Paediatric Department in recent years and those attending the Paediatric Research Unit, Guy's Hospital, a total of 318 *DMD* and 59 *BMD* families. A letter was sent to the 213 families attending the Hammersmith Hospital Clinic at present, explaining the project and offering genetic counselling services. Subsequently, families who had attended the South-East Thames Regional Genetics Centre at Guy's Hospital were also contacted.

Those families consenting to participate were seen, counselled, and blood was taken, where possible, from the index case and his mother, for DNA and CK analysis. Further blood samples were taken from females in the families who required genetic counselling and from males, such as normal brothers, the father, and the uncles of the index case, who could provide phase information for counselling. If there was a positive family history of muscular dystrophy, more extensive family studies were undertaken.

We have personally seen 236 *DMD* and 42 *BMD* families from among the patients attending the Hammersmith Hospital, and the South-East Thames Regional Genetics Centre and the Department of Paediatric Neurology, both at Guy's Hospital. The probands were examined by at least one of the

authors. We took pedigree information and confirmed the diagnosis on the basis of history, physical examination, serum creatine kinase levels, EMG, and muscle biopsy results.⁷ Not all families contained a living affected male who was available for sampling. Blood was taken from 181 boys with *DMD*, 34 males with *BMD*, and 823 relatives, for DNA studies.

CREATINE KINASE ESTIMATIONS

Creatine kinase activity was assayed at the Hammersmith Hospital by the Scandinavian recommended method⁸ and results were based, where possible, on the average of three separate blood samples from each subject. Likelihood ratios were calculated from the values obtained from obligate carriers of *DMD* and those obtained from normal women, and these were combined with the pedigree data using a Bayesian calculation, to give a combined risk for a woman of being a carrier of muscular dystrophy.⁹

RISK CALCULATIONS

Carrier risk estimates combining linkage data with CK levels were calculated either by hand or using the computer programme RISKDNA, which integrates information from DNA markers with pedigree and CK data.¹⁰

DNA ANALYSIS

DNA was extracted from whole blood¹¹ and digested with relevant restriction endonucleases. After electrophoresis and Southern blotting,¹² filters were hybridised with appropriate ³²P labelled DNA probes. Details of the loci tested, the probes used, and the heterozygote frequencies we observed are listed in table 1.¹³

Our strategy for the use of DNA probes for genetic counselling is as follows. For carrier detection, we screened for heterozygosity in a female, often the mother of the proband, using DNA markers in the following order: (1) pERT87-1 and pERT87-15, using an *XmnI* digest. (2) pXJ1-1 and pERT87-15, using a *TaqI* digest. (3) pERT87-8, using a *BstXI* digest. (4) pERT87-1, using *BstNI* and pERT87-8, using *TaqI*. When the woman was found to be heterozygous at a locus defined by one of these probes, this was used for analysis of her family.

Where flanking DNA markers were available, these were used preferentially. However, information using markers pXJ1-1 and pERT87 alone, with an estimated recombination fraction of 0.05 with the *DMD* and *BMD* loci,¹⁵ made substantial differences to carrier risk estimates and was an efficient first screen.

TABLE 1 Details of the DNA probes used.

Locus	Probe	Restriction enzyme	Recombination fraction used for counselling	Observed heterozygote frequency (%)	Reference
<i>DXS41</i>	99-6	<i>Pst</i> I	0.15	40.9	14
<i>DXS164</i>	pERT87-15	<i>Xmn</i> I	0.05	41.5	15
	pERT87-15	<i>Taq</i> I		37.2	
	pERT87-8	<i>Bst</i> XI		40.6	
	pERT87-1	<i>Xmn</i> I		36.2	
<i>DXS206</i>	pXJ1.1	<i>Taq</i> I	0.05	30.3	16
<i>DXS84</i>	754	<i>Pst</i> I	0.16	50.0	17
<i>DXS28</i>	C7	<i>Eco</i> RV	0.12	34.4	18
<i>DXS7</i>	L1.28	<i>Taq</i> I	0.20	40.2	19

Screening of mothers wishing prenatal diagnosis was performed using the above markers, closely linked to *DMD* and *BMD*, and, in addition, the flanking markers 754 and 99.6 (using *Pst*I), C7 (using *Eco*RV), and RC8 and L1.28 (using *Taq*I). Prenatal diagnoses were performed on DNA extracted from chorionic villus samples, using the closest marker (pXJ1.1 or pERT87) informative in the mother, and the two closest informative flanking markers.

Close flanking DNA markers (754, 99.6, and C7) were used preferentially for prenatal diagnosis, but where a woman presented late in pregnancy, prenatal advice was sometimes given using only the closely linked DNA probes pERT87 and pXJ1.1. Risk estimates and the possibility of inaccurate diagnosis using linked DNA markers were fully discussed with the families concerned, and decisions based on this advice were taken in full cognisance of the accuracy of the tests used in each case.

Results

UPTAKE RATES

Our 213 initial letters drew responses from 94 families. Of these, 80 indicated their willingness to participate in the study and 14 families within this group asked for urgent genetic counselling. A further 14 families declined to take part in the studies. Despite this moderate initial response, virtually all of the families who had not replied to the questionnaire, as well as half of those initially declining to participate, were subsequently seen for counselling and testing. An additional 72 families were later recruited, totalling 236 DMD and 42 BMD families.

STRUCTURE OF FAMILIES

The family structures of all 318 DMD and 59 BMD families known to us are shown in tables 2 and 3. A total of 23% of DMD index cases and 32% of BMD cases were familial. In 82% of DMD cases and 78%

of BMD cases, there were close female relatives, other than the mothers of the index cases, who might require genetic counselling.

Of the 278 families seen personally in this study, 28% of 236 DMD index cases and 33% of 42 BMD cases were familial. In these families, the mean number of sibs of the DMD index cases was 1.59 ± 1.34 , and of the BMD index cases 1.84 ± 1.46 .

TABLE 2 Structure of families initially ascertained.

	DMD	BMD
Total clinic population (No of families)	(318)	(59)
Multiple affected males (10 index cases dead)	23%	32%
Isolated case with unaffected brother (10 index cases dead)	33%	24%
Isolated case without brother	41%	44%
Dead isolated case without brother	3%	—
Families personally seen (No of families)	(236)	(42)
Multiple affected males (8 dead)	28%	33%
Isolated case with unaffected brother (7 index cases dead)	30%	28%
Isolated case without brother	39%	39%
Dead isolated case without brother	3%	—

TABLE 3 Female relatives of affected boys.

Total clinic population
318 families with Duchenne muscular dystrophy
61% had sisters
54% had aunts
Total No of sisters/aunts=708
59 families with Becker muscular dystrophy
68% had sisters
49% had aunts
Total No of sisters/aunts=120
Families seen personally in this study
236 families with Duchenne muscular dystrophy
61% had sisters
68.6% had aunts
Total No of sisters/aunts=402
42 families with Becker muscular dystrophy
47.6% had sisters
59.5% had aunts
Total No of sisters/aunts=84

CLINICAL DETAILS (TABLE 4)

None of the affected boys had dysmorphic features. Many boys with DMD had speech delay and required specific therapy for this. Mental retardation was found in 29% of DMD (table 4), but was not found in BMD, in accordance with previous experience.¹ Of DMD boys with mental retardation, the average age at which ambulation was lost was 9.8 ± 1.54 years old; in the group with normal IQ, this was 9.6 ± 1.5 years old. Fifteen boys lost ambulation between the ages of 13 and 17 years, and were thus in the intermediate range between DMD and BMD.

In 18 pairs of brothers with DMD where intelligence was assessed, two pairs of affected boys both had mental retardation, while the 16 other pairs of brothers were all of normal intelligence. One family of three brothers (aged four years and younger) with muscular dystrophy all had speech and developmental delay. Their uncle, who had DMD, had an IQ of 50.

The clinical courses in two or more brothers, or more distant relatives, affected with DMD or BMD, were similar in 28 out of 30 families where detailed information was available.

In one family the uncle of the index case lost ambulation at 17 years of age and was alive, in a wheelchair, at 40 years of age, while the index case has a disorder more suggestive of DMD. However, as he is still only seven years old, his clinical course cannot be predicted. One pair of brothers had slightly discordant courses, in that one brother walked at 18 months and ceased to walk at 12 years, while the other walked at 12 months and ceased to walk at 16 years. Both had speech delay. Even in these cases, the discrepancies noted were not marked.

In an additional pair of brothers there was a striking difference in clinical symptoms, where one brother had classical DMD and ceased to walk at six and a half years, but the other had minimal proximal hip girdle weakness at 15 years, despite a high serum CK level. The mildly affected brother showed

minimal myopathic changes on muscle biopsy and chromosome analysis revealed the karyotype 47,XXY, so that his symptoms were presumed to be features of the manifesting DMD heterozygote (A Walker, 1986, personal communication).

CARRIER RISK ESTIMATES USING LINKAGE

The way in which carrier risk estimates have been altered by the use of linkage analysis in the 113 women where useful information was obtained is shown in the figure. So far, we have studied 52 sisters of affected boys, of whom 28 were sisters of sporadic cases. In only two of these (in families of sporadic cases) was no alteration of risk estimate possible using linkage analysis. A total of 43 aunts of affected boys has been studied, of whom 38 were aunts of sporadic cases, and in only two (both in families of sporadic cases) was no useful linkage information obtained. The carrier risks of 69 obligate carriers were, of course, unaltered by linkage analysis. As has been previously reported,²⁰ linkage analysis generally correlates well with high or low risk estimates using pedigree and CK data, and tends to move the risk estimates towards the extremes of the risk range, leaving fewer women in the ambiguous middle range of risks between 20 and 80%. Of the 113 women represented in the figure, 37 were in this risk range before linkage analysis. After adding linkage data to the risk calculation, only seven women remained in this middle range.

FAMILIES OF SPORADIC CASES

In the 171 (72.5%) DMD and 28 (66.7%) BMD families where the probands were isolated cases, linkage analysis significantly altered the risk to mothers of probands in three specific situations. In four cases, the proband was found to have a DNA deletion (detected by non-hybridisation of the patient's DNA to subclones of pERT87 or pXJ1.1²¹) and his mother was heterozygous at the site detected by the marker for which her affected son was deleted. In these cases, the mother's risk is greatly reduced, as she is clearly not carrying the deletion which is presumed to cause the disease in her son. In practice, because of uncertainty about the precise relationship between the DNA deletion and the DMD mutation, these women were given a low carrier risk estimate, but with some reservations. In five families, the affected boys were found to have inherited the same alleles linked to DMD as their normal brothers, which markedly reduced the likelihood that the mother was a carrier. Conversely, if an affected boy had inherited different alleles from his unaffected brother, this somewhat increased the mother's carrier risk estimate (in five mothers studied). Information about the grandparental ori-

TABLE 4 Clinical details of affected boys.

	DMD	BMD
% learning to walk below age of 18 months	50% n=188	62.8% n=43
Mean age of loss of ability to walk (year)	9.5 ± 1.75 n=148	$23.2 \pm 11.2^*$ n=6
Mean age at first recorded use of sentences (year)	2.5 ± 1.12 n=166	2.3 ± 0.85 n=32
Mental retardation (ESN or IQ below 80)	28.7% n=130	0/17

*36 BMD patients are still ambulant, 26 of whom are below the age of 16 years. The diagnosis of BMD in these cases is based upon their relatively mild clinical features.

FIGURE The effects of linkage analysis on carrier risk estimates. (a) 70 women from families of sporadic cases of BMD or DMD in whom linkage analysis altered carrier risk estimates. (b) 29 women from families of familial cases of BMD or DMD in whom linkage analysis altered carrier risk estimates. (c) 14 women in whom carrier risks were altered, from families of BMD or DMD cases with DNA deletions. Each arrow represents a woman's carrier risk estimates. The arrow starts at her risk estimate using CK and pedigree data and ends at the risk estimate incorporating DNA linkage data.

gin of the alleles inherited by the affected boy had a small effect on the mother's carrier risk estimate in eight cases; thus, her risk was slightly reduced if the affected boy had inherited the grandpaternal allele. The proportion of 11% of mothers of isolated cases whose risk estimates were altered with the use of linkage analysis could rise to about 28% when linkage studies are completed.

Although carrier risks to mothers of sporadic cases were not often greatly altered, linkage information was used to alter carrier risk estimates for sisters and aunts of the proband in these families. We used linkage calculations to alter the carrier risks of 53 sisters of affected boys.²² In 15 families where linkage information indicated that the isolated affected boy had inherited his grandpaternal

alleles linked to the *DMD* locus, the probability was increased that his disease had arisen as a new mutation, either in the grandpaternal or the maternal gamete. Consequently, the risk that the grandmother was a carrier was reduced and this, in turn, reduced the carrier risk estimates for 13 aunts. If, however, the affected boy had inherited the grandmaternal allele, as in nine of our families, the carrier risks to his aunts depended upon whether or not they had inherited from their mother the same allele as the mother of the affected boy. This was the case with seven aunts.

ATTITUDES TO PREGNANCY

Eleven women with carrier risks higher than 20% decided to have no further children (many opting for

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sterilisation). This is probably an underestimate, because women making this decision do not often seek further genetic advice.

Table 5 shows the decisions of women at risk of being carriers regarding pregnancies before the availability of prenatal diagnosis using linkage analysis.

Women perceiving their carrier risk to be low (below 10%) often elected to take the small risk of having an affected son without prenatal diagnosis, but most women with carrier risks above 30% elected to have fetal sexing and termination of pregnancy if the fetus was found to be male. Occasional exceptions are found: in our series two sisters at very high risk of being carriers both elected to continue their pregnancies without any form of prenatal diagnosis.

We have been asked for prenatal diagnosis or genetic counselling using linkage analysis in relation to 17 pregnancies in 15 women (table 6). Three women (cases 9, 10, and 12) who had their carrier risk estimates substantially reduced by DNA analysis from 50 to 5%, 25 to 2%, and 20 to 2%, respectively, elected to continue their pregnancies

without prenatal diagnosis. Two women (cases 13 and 15) had prior carrier risk estimates, on pedigree and CK data, which they considered low enough (10% and 0.5%) for them to continue their pregnancies without prenatal diagnosis, but DNA studies provided additional reassurance by reducing their carrier risk estimates even further.

Prenatal diagnosis was requested by seven women at high risk of being carriers who were prepared to take a 5% or smaller risk of having an affected son. Three pregnancies found to be female were continued. Four carrier women (cases 1, 2, 5, and 11) elected to continue pregnancies found to be male with low (5% or less) risks of being affected, whereas three women (cases 6, 8, and 14) terminated pregnancies found to be male with higher (48%, 28%, and 8%) risks. It is interesting that case 2 was not prepared to take a 5% risk of an affected son in her first pregnancy, but was prepared to do so in her second. Case 1 requested prenatal diagnosis in her first pregnancy, but was not heterozygous for any of the markers available at that time. In her second pregnancy, however, one was found, and she is continuing a monochorionic twin pregnancy with

TABLE 5 *Decisions about 36 pregnancies in carrier women before the availability of linkage analysis for prenatal diagnosis.*

Intention	Risk						Total
	0-10%	10-20%	20-30%	30-50%	50-75%	75-100%	
Terminate male pregnancy	1	2	2	7	1	7	20
Keep male pregnancy	8	2	1	0	1	2	14
Not sure (kept female pregnancy)			1			1	2

(Two of these were in families with Becker muscular dystrophy.)

TABLE 6 *Use of linkage analysis in 17 pregnancies.*

Case No	Carrier risk (%)	Relationship to affected male	Sex of fetus	Risk estimate after prenatal diagnosis (%)	Outcome
1	100	Aunt	M	Not informative	Terminate
			M twins	5	Continue
2	100	Sister	F		Continue
			M	5	Continue
3	100	Sister	F		Continue
4	100	Sister	F		Continue
5	100	Niece	M	5	Continue
6	50	Sister (BMD)	M	43	Terminate
7	30 ↓ 3	Sister	M		Terminate
8	50 ↓ 30	Mother	M	±28	Terminate
9	50 ↓ 5	Sister	?		Continue
10	25 ↓ 2	Sister (severe BMD)	?		Continue
11	20 ↓ 2	Sister	M	±0.1	Continue
12	20 ↓ 2	Aunt	?		Continue
13	10 ↓ v low	Aunt	?		Continue
14	8-9	Sister	M	±8	Terminate
15	0.5 ↓ v low	Cousin	?		Continue

Risks in *italics* obtained by combining linkage analysis with previously available carrier risk estimates.

an estimated 5% risk of DMD. At present, 11 of these pregnancies are continuing.

Discussion

The clinical features of the population of boys with DMD and BMD in this study were not unusual, and there was no case which showed dysmorphic features. No difference was found between the ages at which the DMD boys with mental retardation and those with normal intelligence lost ambulation. Heterogeneity has been suggested in the past, using these parameters, by some authors,²³ but not by others.²⁴ The features of DMD and BMD were seen as a spectrum, with some BMD cases quite severely affected. There was considerable clinical resemblance within families, both in the presence or absence of obvious mental handicap and in the general severity of the muscle disease. In the only family in which the disease severity in two brothers differed markedly, one was found to have the chromosome constitution 47,XXY and was presumably a manifesting heterozygote for DMD.

There was an overwhelmingly positive response to our approaches to families for cooperation in family studies, and we have become increasingly involved in providing a genetic counselling service for them. We have already performed restriction fragment length polymorphism (RFLP) analysis on 215 affected males and 823 relatives, and we estimate that there are potentially a further 350 samples to be taken, if we are to complete our analysis of potential carriers in these families. We will therefore analyse in total about 1400 samples from 278 families. This gives an estimate of the workload involved in applying linkage to the analysis of X linked disorders for genetic counselling, which may be of value in planning further service development.

Our experience with genetic counselling suggests that linkage information provides further reassurance to women at low carrier risks, who may then undertake pregnancies without prenatal diagnosis. Women with higher carrier risks may still prefer to have pregnancies terminated if found to be male, but many may continue a pregnancy if prenatal tests show a male to have a 5% or lower risk of being affected. In the absence of disease specific prenatal tests, reproductive compensation after termination of male pregnancies probably had the dysgenic effect of increasing the numbers of carrier daughters born. Parents of affected boys appear to have had approximately the same number of children as the general population. The average size of the families we have studied, including the probands, was approximately 2.7. The Registrar General's statistics give family size as varying between 2.0 and 2.3

since 1928. However, our sample includes some sibships of older generations and a few families from outside the UK.

Because the current linkage estimates have error rates of the order of 2 to 5%, the most dramatic qualitative change in carrier risk estimates is seen in women with prior carrier risks in the low to intermediate range. These women had low, but not negligible, risks which they were unwilling to run. Dropping these risks by a factor of 20 altered their decisions about whether to undergo pregnancies without prenatal diagnosis. Linkage studies significantly alter the carrier risks of about 11% of mothers of isolated cases. In those families where DMD is associated with deletion of a probe site, more accurate counselling of mothers is sometimes possible.

As linkage information becomes more accurate, it is hoped that it will allow carrier women to have families without the fear of having an affected child and without the guilt consequent upon the termination of pregnancies which could have been normal. However, we remain unable to predict the two thirds of cases of DMD which present with no previous family history.

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