

A register based system for gene tracking in Duchenne muscular dystrophy

A P READ, L KERZIN-STORRAR, R C MOUNTFORD, R G ELLES, AND R HARRIS

From the Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH.

SUMMARY A total of 102 families with Duchenne muscular dystrophy has been studied with linked DNA polymorphisms as an aid to estimating carrier risks for female relatives. Early work using probes RC8, L1.28, and pXUT23 gave very little clinically useful information because of the high recombination rates between these probes and Duchenne muscular dystrophy and the low proportion of women who were heterozygous. Clinically useful results were obtained using probes 99-6, 754, and particularly pERT87. Examples are given of deductions which can be made using these probes. The importance of a genetic register is stressed as a tool for long term contact with the families and other professionals.

In Manchester, clinical genetic services for Duchenne muscular dystrophy (DMD) are organised through a genetic register. The primary purpose of the register is to counsel all females at risk of being carriers of DMD in the North West Region (population 4 million). The register was set up some years ago, before gene tracking became possible, but in anticipation of such a development. We have aimed at complete ascertainment of all DMD families in the region.

Although there has been continuous development in gene tracking for DMD, we can divide our work into three cycles. First we used L1.28¹ and RC8¹ or pXUT23²; RC8 and L1.28 both show polymorphic bands with *TaqI* and can conveniently be used in a mixed hybridisation. In the second cycle we used 99-6³ and 754⁴ which again can be used in a mixed hybridisation with a *PstI* digest. The third cycle is based on the pERT87 probes.⁵

In common with other groups, we felt that attempts at prenatal diagnosis using the earlier generations of probes would not be justified. Carrier risks could be modified in most informative families, but only in a few cases would the change be clinically significant, that is, large enough to persuade a woman at risk to change her mind about fetal sexing. The maximum change achievable with a given

pedigree structure and probe(s) can be estimated. With pedigrees of any complexity a programme such as MLINK⁶ must be used, but for simple situations the RFLP data can be incorporated as conditional probabilities into a Bayesian calculation. Pembrey *et al*⁷ give examples. The table shows the change achievable in some simple cases.

The probes RC8, L1.28, and pXUT23 used in the first cycle of testing all show 15 to 20% recombination with DMD.⁸ The calculations in the table and others suggested that these probes would yield clinically useful information only in families with obligate carriers. The second cycle probes, 99-6 and 754, show about 15% and 10% recombination respectively with DMD.⁸ Families without obligate carriers might be helped if they were informative for bridging markers (99-6 and 754 or L1.28). Our study of these families was not complete when the pERT87 probes became available. Unlike previous probes, pERT87 is a family of probes detecting polymorphisms with several different enzymes (pERT87-1 with *XmnI*, *EcoRV*, and *BstNI*; pERT87-8 with *TaqI* and *BstXI*; pERT87-15 with *TaqI*, *XmnI*, and *BamHI*). Over 90% of women are heterozygous for one or more of these polymorphisms. With a recombination fraction of only 5%,⁹ a very high level of polymorphism, and the possibility of finding deletions, these probes are potentially useful in almost all families.

TABLE Use of RFLPs to estimate the risk of whether a girl is a carrier of DMD: likelihood ratios in fully informative pedigrees.

Pedigree structure	Probes	Approximate formula	Likelihood ratio when probe(s) fully informative			
			$\theta=0.20$	$\theta=0.15$	$\theta=0.10$	$\theta=0.05$
A	Single probe	$\frac{1-\theta}{\theta}$	4.0	5.7	9.0	19.0
	Bridging probes	$\frac{1-\theta_A\theta_B}{\theta_A\theta_B}$	24.0 (0.4)*	43.4 (0.3)*	99.0 (0.2)*	399.0(0.1)*
B	Single probe	$\frac{(1-\theta)^2+\theta^2}{2\theta(1-\theta)}$	2.12	2.9	4.6	9.5
	Bridging probes	$\frac{1-2(\theta_A+\theta)+2(\theta_A+\theta_B)^2}{4\theta_A\theta_B(1-\theta_A)(1-\theta_B)}$	5.1 (0.8)*	8.9 (0.6)*	21.0 (0.4)*	90.9 (0.2)*

*Probability no inference can be made because there is a marker recombinant.

Pedigree structure A: daughter of a phase known obligate carrier.

Pedigree structure B: daughter of a phase unknown obligate or possible carrier, where phase is inferred from a single son. Where more than one son is available, the likelihoods approximate to those of structure A, but an increasing proportion of pedigrees are uninformative because of marker-marker recombination.

Results

A total of 102 families with DMD has been studied to date. These include 46 with one or more available obligate carriers. Forty-two families on the register have not yet been studied because of poor structure (for example, no living obligate carrier or affected boy) or poor access to the potentially informative branches of the family.

SCREENING WITH L1.28 AND RC8

We screened 51 obligate carriers in 36 families. Twenty were informative with L1.28 and 14 with RC8, but only two were doubly informative. One of these two had one daughter who was apparently not a carrier; the other had only sons. Forty-two of the obligate carriers were rescreened with pXUT23. Ten were heterozygous, but only one was also informative with L1.28, giving bridging markers. She had one daughter who, however, showed a recombination between pXUT23 and L1.28.

SCREENING WITH 99-6 AND 754

We screened 53 obligate carriers in 38 families. Thirty were informative with 99-6 and 27 with 754; 14 were doubly informative. When the results with RC8, L1.28, pXUT23, 99-6, and 754 were combined, together with a few results with OTC, 28 obligate carriers were informative with bridging markers, and they had 44 daughters at risk. The next stage in this work was to screen families without

obligate carriers with 99-6 and 754, and to test obligate carriers with OTC and D2 to find bridging markers. We were starting this phase when the pERT87 probes became available and we laid this work aside to concentrate on screening with pERT87.

SCREENING WITH PERT87

Our policy has been to make one *TaqI* filter for mixed hybridisation with 87-8 and 87-15 and, if this fails to reveal heterozygosity, to make an *XmnI* filter and hybridise successively with 87-15 then 87-1. This procedure has yielded very much more useful information per digest than in the previous cycles.

Testing affected boys revealed deletions in five of 62 families. Two were familial and three sporadic. Four were deleted for all pERT probes and one familial case was deleted for 87-8 but not 87-15.

Obligate carriers gave informative polymorphisms in 36 of 48 tested; two of the 12 non-informative carriers are known to be hemizygous deletion carriers. None of the uninformative carriers has yet been tested for all eight polymorphisms. The informative or hemizygous carriers have 57 daughters at risk.

Many of the families without obligate carriers are still being worked up. In three cases we have found deletions and in several others a sporadic affected boy has inherited the same pERT allele as two healthy brothers, making it unlikely that his mother is a carrier. These cases are being tested with bridging markers.

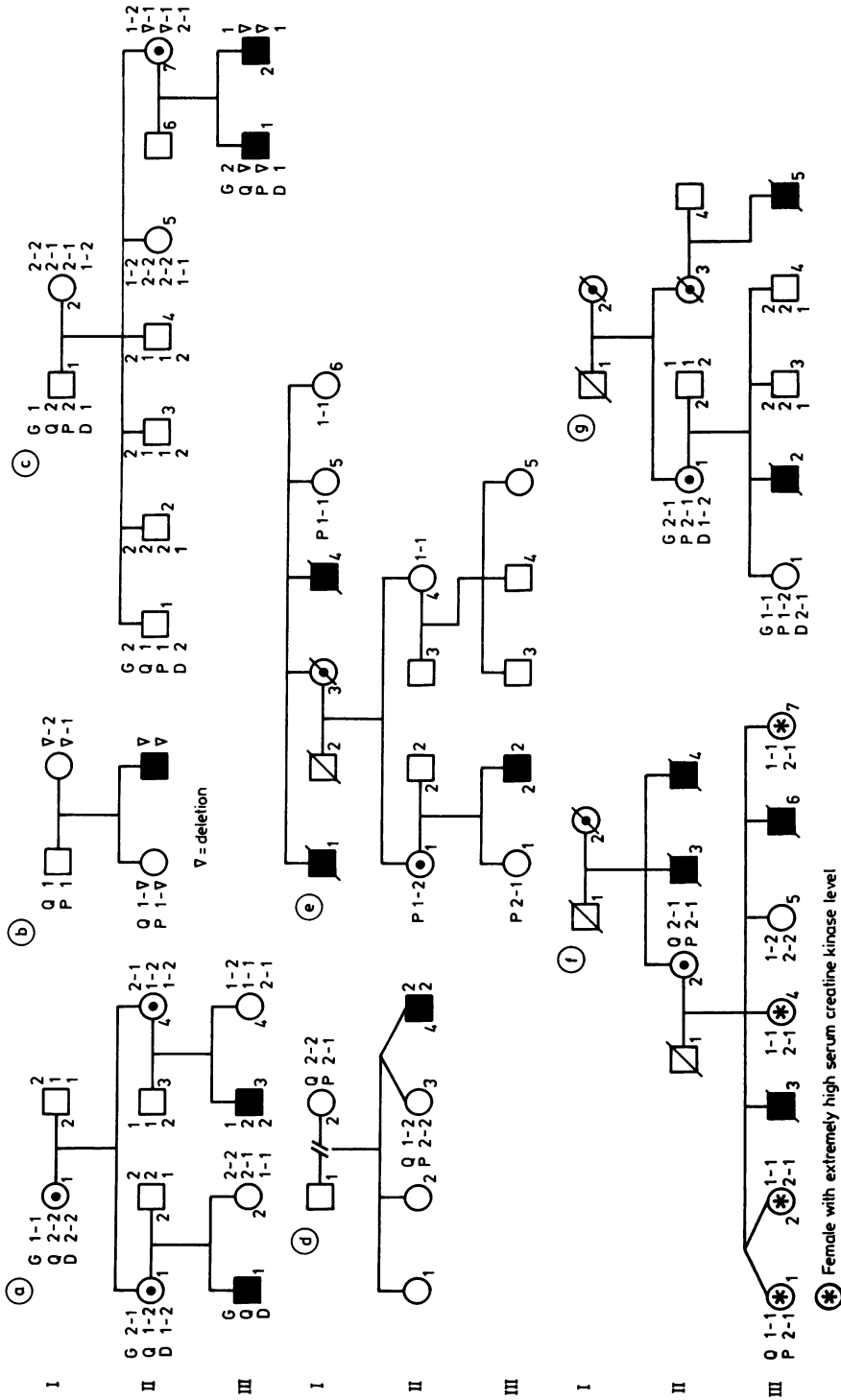


FIGURE Seven families informative for DNA polymorphisms linked to the Duchenne muscular dystrophy locus. (a) Family R.279. (b) Family MS.16. (c) Family R.306. (d) Family R.354. (e) Family R.028. (f) Family R.047. (g) Family R.062. 1, 2 = codes for alleles. Key to loci: D = DXS84 detected with probe 754; G = DXS41 detected with probe 99-6; P, Q = DXS164 detected with probe pERT87-8 (P) or pERT87-15 (Q). See text for details.

The following families (figure) illustrate the application of the pERT87 probes.

(a) *Family R.279*. Subjects II.1 and II.4 are phase known obligate carriers, informative for 99-6, pERT87-15 (*TaqI* polymorphism), 754, and L1.28. Their daughters III.2 and III.4 each inherited the grandpaternal X chromosome. Based on pERT87 alone, their carrier risk is 5% and based on 99-6 and 754 it is 1.5%. Using the bridging markers, pERT87 gives added confidence that there has not been a double recombinant, so that the final risk from the RFLP data is below 1%.

(b) *Family MS16*. The sporadic affected boy is deleted for pERT87-8 and 87-15. His sister shows only allele 1 of the 87-15 *TaqI* polymorphism, while his mother shows only allele 2. Both must be carriers of the deletion.

(c) *Family R.306*. The two affected boys are deleted for pERT87-8 and 87-15. Their grandmother (I.2) is heterozygous for pERT87-8, pERT87-15, and 754. Both of her X chromosomes can be found in her healthy sons (II.2, II.3, II.4), suggesting that she is not a carrier. Her obligate carrier daughter II.7 is apparently homozygous for pERT87-8 and 87-15, but has inherited no pERT87 allele from her father. Paternity was confirmed with HLA; 754 and L1.28 results confirm that the affected boys have the grandpaternal X chromosome. One of them (III.1) is recombinant for 99-6. Here we see a deletion arising de novo in a male, showing that recombination is not a necessary part of the mechanism generating deletions. In practical terms, the sister II.5 can be reassured she is not a carrier, and the obligate carrier II.7 can be offered prenatal diagnosis in any future pregnancies.

(d) *Family R.354*. The sister (II.3) of the sporadic affected boy requested carrier testing and possible prenatal diagnosis. Their father was not available. This pedigree illustrates the value of being able to detect multiple polymorphisms with one probe. The carrier risk was established using a marker (pERT87-8 with *TaqI*) for which the mother was heterozygous but the daughter was homozygous, obviating the need to obtain the father's DNA. Prenatal diagnosis could use the *TaqI* polymorphism of pERT87-15, for which the daughter is heterozygous but the mother is homozygous, so that the maternal allele can be identified unambiguously.

(e) *Family R.028*. The family structure is unfavourable, with both grandparents dead and only one boy to establish phase. Using the pERT87-8 *TaqI* polymorphism, subject II.1 is heterozygous, with probable phase DMD-2. Her sister (II.4) is homozygous 1-1. Provided that both women have the same father, he must have had allele 1 and the mother I.3 was heterozygous 2-1. The obligate carrier II.1 inherited

allele 2 with DMD from her mother as expected, and her sister II.4 is unlikely to carry the DMD gene. Subject I.3 is inferred to be heterozygous 2-1, with phase DMD-2, but her two sisters I.5 and I.6 have been tested and are homozygous 1-1. The sisters I.5 and I.6, who have four daughters and several granddaughters, are therefore unlikely to be carriers. Thus, despite a poor pedigree structure, the carrier risks for all other branches of the family have been substantially reduced. Quantification is difficult because of the assumption about paternity.

(f) *Family R.047*. The structure is unfavourable, with an obligate carrier but no surviving sons to establish phase, and the father of the girls at risk is dead. Of the five daughters, all except III.5 have very high CK levels and two have muscle weakness. The mother is informative for the *TaqI* polymorphisms of pERT87-8 and 87-15. In each case the four girls with high CK levels have all inherited one allele from their mother and the daughter with a low CK level has inherited the other. Four daughters are homozygous for 87-15 but heterozygous for 87-8, while the fifth is heterozygous for 87-15 but homozygous for 87-8. Therefore, assuming that they have the same father, his 87-8 and 87-15 genotypes can be inferred, allowing prenatal diagnosis to be offered to the four high risk girls. With five children and one parent, it should be possible to use the minisatellite probe of Jeffreys *et al*¹⁰ to show that they have the same father. We are working on this.

Information systems

We quickly discovered that organising the flow of information between the clinicians and the laboratory was not a trivial task. Organising the workload is much more complex for a laboratory offering a service than for a purely research laboratory. It is highly inefficient to work up one family fully before moving on to another. As far as possible, blood samples are taken at the regular annual review of each family. Samples are best processed in batches of a size to fill a gel. Results from one sample are seldom meaningful until combined with other results from the family. We are dealing with several diseases apart from DMD, and our data files now include over 4000 patients, 2500 DNA samples, and 3000 RFLP results. We have devoted considerable effort to devising a system to handle this data efficiently, robustly, and confidentially. Our system runs on IBM PC-AT microcomputers under dBaseIII. It maintains linked data files of patients and RFLP results and an inventory of DNA samples. We have operated this system for about 18 months without trouble, and have not noticed any degradation of performance as

the volume of data rises. Copies of the programmes are available on request.

Discussion

A particular feature of the DMD work in Manchester has been the use of the genetic register. We see this as the natural approach to taking long term responsibility for counselling these families. The register has advantages for the families: they can consider what tests they would want without the pressure of a pregnancy. This is particularly important where family members may first realise they are at risk as a result of contact with the genetic services. Ideally, in advance of any pregnancy, the pedigree will have been worked up, CK levels estimated twice, DNA samples collected, and appropriate females checked to see whether they are informative. Then, prenatal diagnosis need not be attempted if RFLP analysis shows that the mother is unlikely to be a carrier or is uninformative for available probes.

The long term contact which follows from the register approach is particularly important with the rapid advances in this field. Our families are aware that all risks are provisional pending further progress. All families are reviewed annually but, increasingly, girls at risk are contacting us to let us know that they are planning marriage or a pregnancy, so that they can be counselled using the latest information available. This arrangement is also helpful to the laboratory for planning priorities. The register also provides an important link with general practitioners, who are becoming increasingly aware of the implications of the new technology.

At the end of the first round of testing with RC8, L1.28, and pXUT23, only one girl at risk had seen her carrier risk substantially changed by the RFLP results. This was due to the frequent homozygosity, particularly with probes RC8 and pXUT23, the high recombination rate, and some bad luck that so few carriers were doubly informative. Compared to this, the impact of pERT87 has been revolutionary. For the first time we are able to offer useful risk estimations to almost all the families on the register.

With all its advantages, pERT87 shows one disadvantage: it cannot be used as one of a pair of bridging markers because it is not clear that DMD mutations lie on the same side of the pERT87 (*DXS164*) locus in all families. The dilemma is therefore either to use pERT87 as a single marker, highly informative but with 5% recombination, or to use other, definitely bridging markers such as 754 and 99-6 and not take advantage of pERT87. Ideally, counselling would be based on bridging markers,

with pERT87 as a check against double recombination. Unfortunately, it often happens that many probes must be tried before informative bridging markers are found and then there may be recombination between them. Family R.062 (figure (g)) shows the problems this can cause. Subject III.1 has a recombination between 99-6 and pERT87, but is non-recombinant between pERT87 and 754. Thus, the reward for the extra work of seeking bridging markers can be small. We also feel that the uncertainties of DMD at present are such that it may be prudent when counselling not to quote extremely low carrier risks. Our working practice has therefore been to base carrier risks on pERT87 data alone. For prenatal diagnosis, bridging markers would be used wherever possible in addition to pERT87, despite the difficulties when there is recombination between the bridging markers.

Gene probes for DMD have now reached the stage where they are significantly affecting the counselling of females at risk. Where there is a deletion, counselling and prenatal diagnosis are generally easy, although a difficulty arises if the mother of a boy with a deletion is heterozygous. Can she be assured she is not a carrier? Could some of these mothers carry a premutation and be at risk of having further affected boys? Despite these uncertainties, it has been gratifying to see several sisters of DMD boys decide their risks are low enough to embark upon pregnancy without plans for prenatal diagnosis.

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Correspondence and requests for reprints to Dr A P Read, Department of Medical Genetics, St Mary's Hospital, Hathersage Road, Manchester M13 0JH.