Molecular analysis and diagnosis of Duchenne muscular dystrophy

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive condition which affects 1 in 2,500 males [1]. Affected males are wheelchair bound by the age of twelve and die in the third decade of life. A clinically milder course is followed in Becker muscular dystrophy (BMD) where the boys lose the ability to walk much later and on occasions have a normal lifespan. Until six years ago, no reliable carrier detection or antenatal prediction was possible for these diseases although a high level of creatine kinase in the serum of females at risk was a useful indicator of carrier status. With the isolation of closely linked DNA markers detecting restriction fragment length polymorphisms (RFLPs) more reliable carrier detection and prenatal diagnosis could be offered to some families [2].

Recently, DNA sequences have been isolated (Fig. 1) which are deleted in 10 per cent of DMD and BMD patients (pERT 87 [3], XJ1.1 [4], HIP25 [5]). This work has led to the identification of the gene sequence mutated in the disorders [6–9]. This short review describes the characteristics of this gene, its possible function, and its use in the carrier detection and prenatal diagnosis of DMD and BMD.

Characteristics of the DMD gene

The DMD gene is the largest gene so far identified in the human genome. The gene is localised across the Xp21 band on the short arm of the X chromosome and encodes on mRNA of 14 kb (Fig. 1). The coding region corresponding to this mRNA is divided into at least 60 separate segments (exons) spread over at least 2,000 kilobases (2 Mbp) of genomic DNA. The enormous size of the DMD locus may in part explain the high new mutation frequency (7×10^{-4}) which is one of the highest observed for genetic diseases in man [10]. Figure 1 shows the map of Xp21 together with the localisation of adrenal hypoplasia (AHC), glycerol kinase deficiency (GK), chronic granulomatous disease (CGD) and ornithine transcarbamylase deficiency (OTC).

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The DMD gene shows a large degree of conservation in evolution. Analysis of the sequence suggests a structural role for the corresponding protein [11,12] although its

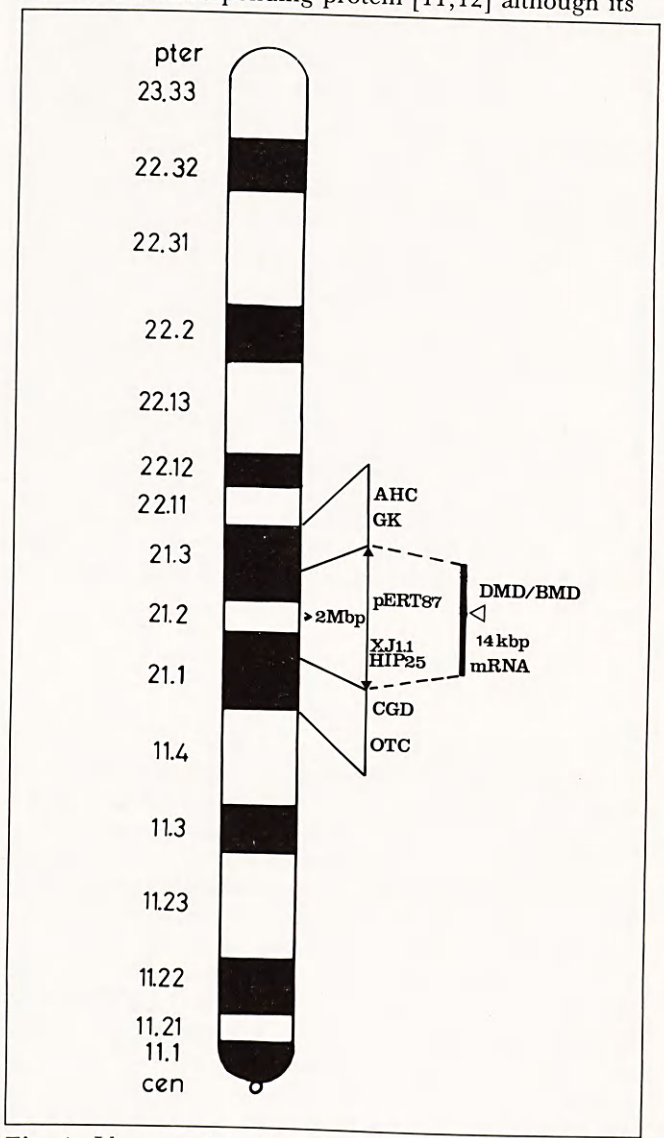

Fig. 1. *Ideogram of the short arm of the human X chromosome showing the localisation of the DMD gene.*

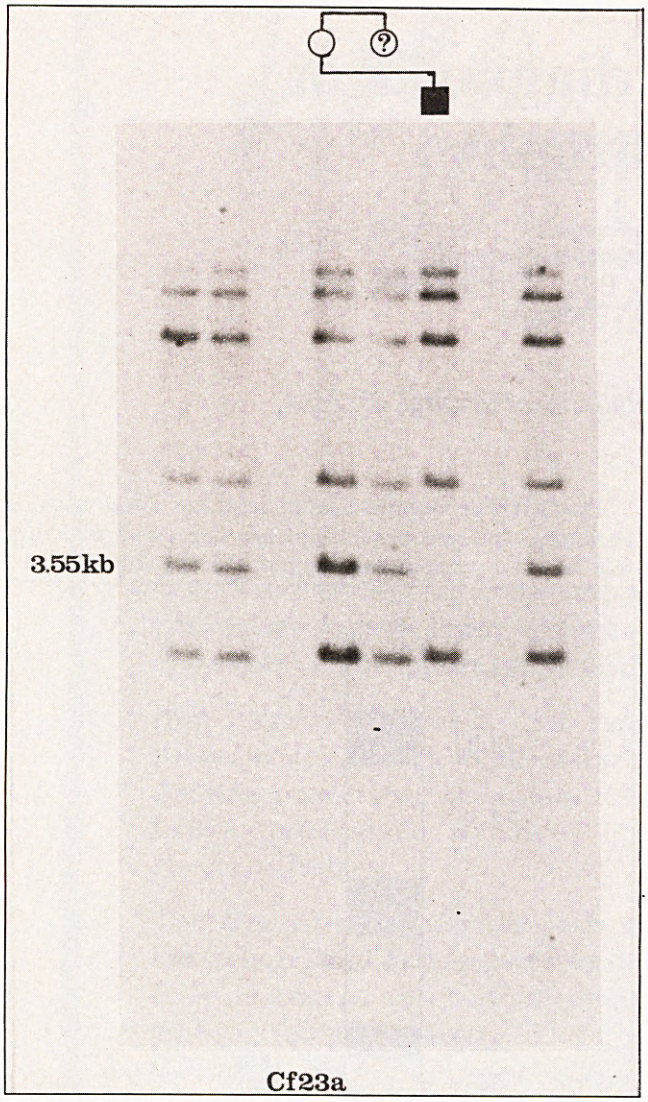

Fig. 2. *Southern blot analysis of a BMD family. Pst I digests hybridised to the cDNA sequence Cf23a. Son ■; mother, ○; aunt ?*

exact function in the cell remains to be determined. Comparison of the DNA sequence with that known for other muscle proteins shows that the DMD gene shares homologies with alpha actinin, a protein which is a normal component of actin filaments in smooth and skeletal muscle and may be involved in both cross-linking F-actin within the filaments and in connecting filamentous elements of the cytoskeleton to the cell membrane [13]. The DMD gene is expressed in both fetal and adult muscle cells and there is so far no evidence for differential splicing of the transcript during development [12]. Thus, the difference between DMD and BMD phenotypes cannot be explained in the context of specific fetal and adult proteins. It may be that in DMD no protein is synthesised whereas in BMD a mutant but nevertheless partially functional protein is produced, leading to a milder phenotype. A detailed analysis of several of the mutations is required to answer this question.

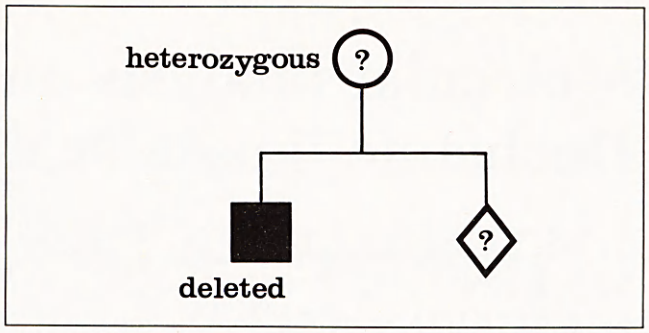

Fig. 3. *Hypothetical situation for genetic counselling.*

Approximately one-third of DMD boys are mentally retarded and yet there appears to be no correlation between the IQ and the portion of the gene deleted in patients. The only general rule is that patients deleted for the whole locus are mentally retarded. These observations may indicate that there is an adjacent gene in the region, the expression of which is modified by the deletions.

Diagnosis of DMD and BMD

Recently, a region of the gene has been identified which is very prone to deletion [7, 9]. One cDNA probe, 1.5 kb in length, detects deletions in 50 per cent of BMD patients. A typical blot with this probe is shown in Fig. 2 where the BMD patient lacks a band in the Pst I digest at 3.55 kb. Neither his mother nor his aunt show a single dose for this band although this is quite difficult to visualise on the blot shown. This result, together with the lack of a previous family history, suggests that this boy is affected by a new mutation.

The ascertainment of carrier status by gene dosage can often be very difficult because of the non-uniformity of the blotting of the gel. However, over 90 per cent of these BMD deletions show endpoints in the same region of the DMD gene. If these breakpoints lie close together, it should be possible in the near future to develop a probe bridging this part of the locus which would directly detect all the deletion breakpoints. In these cases the carrier status of the female at risk will be evident by the presence of a changed band in the gel. This approach will be valuable in those cases where the proband is dead since, if his mother has a changed band then all other females in the pedigree can be assessed for the same abnormal gel profile.

The situation for DMD patients is similar to that for BMD except for the fact that only 30 per cent of the deletions begin in the same region of the gene. A 0.9 kb cDNA probe which lies adjacent to the BMD deletion probe described above can detect a deletion in approximately 50 per cent of the DMD patients.

One of the complicating features of carrier status determination in these diseases is presented in Fig. 3. The son was found to possess a deletion but his mother was heterozygous for an RFLP at this locus, indicating the

absence of the deletion. At first sight, one would conclude that the affected male is a new mutation and that the risk for future affected pregnancies is very small. However, it is now clear that further affected offspring are sometimes produced as a result of germline mosaicism. This mosaicism has been demonstrated in both the male and female germline and may occur in as many as 10 per cent of apparent cases of new mutations [14, 15]. In all these situations, future pregnancies should be screened for the presence or absence of the deletion.

Considering the locus as a whole, it is now possible to diagnose directly more than 60 per cent of DMD and BMD patients by deletion analysis. This greatly facilitates the work of the laboratory and in most cases makes large, expensive and laborious family linkage studies unnecessary. Studies currently being carried out on the function of the protein should lead to a better understanding of the disease and perhaps to improved treatment which might eventually replace the need for antenatal diagnosis.

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