

Dystrophin expression and genotypic analysis of two cases of benign X linked myopathy (McLeod's syndrome)

N D Carter, J E Morgan, A P Monaco, M S Schwartz, S Jeffery

Abstract

DNA extraction and Southern blot analysis of two cases of McLeod's syndrome showed restriction fragments identical to normal controls using probes from the Xp21 (1-2) region, in contrast to striking deletions found in two other McLeod phenotypes studied in the USA. The McLeod locus is adjacent to Duchenne muscular dystrophy (DMD) and dystrophin immunocytochemistry showed that expression is normal in muscle from the two McLeod cases in spite of the mild DMD-like myopathy.

McLeod's syndrome is a rare, X linked condition in which there is pronounced red cell acanthocytosis and myopathy.¹ Muscle biopsies from patients with the syndrome show an active muscle disease, but there is no clinical evidence of functional muscle abnormality.² Deletion analysis has suggested that the McLeod locus is probably located between that for the much more severe myopathy Duchenne muscular dystrophy (DMD) and chronic granulomatous disease (CGD).¹ Studies on males with McLeod's syndrome showed that two related subjects had a deletion detectable at the DNA level at Xp21 in the expected position.¹ Subjects with a combination of CGD and DMD showed large deletions, but other patients, who had McLeod's syndrome only, did not have a deletion

in the same area. Red blood cells from McLeod subjects have been found to lack an immunological marker on a 37 kd protein.³

Since McLeod's syndrome is very rare (approximately 1 per 50 000 to 100 000 of the population), few cases have been studied by DNA analysis and muscle biopsy. We report here an analysis of two unrelated British McLeod males at the DNA level and describe for the first time the distribution of dystrophin in biopsies from the affected males.

Materials and methods

SUBJECTS

The two males with the McLeod phenotype originally

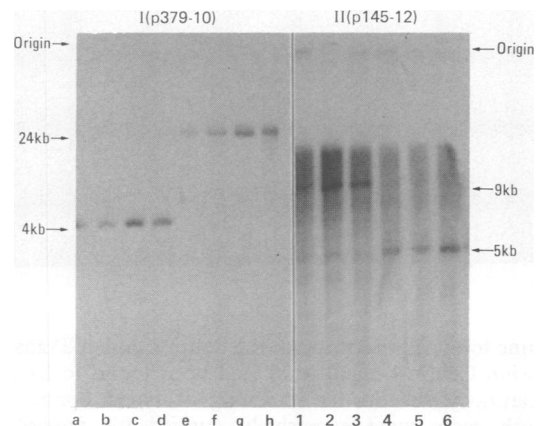


Figure 1 Southern blot of restriction digests of DNA from McLeod males and controls. (Left) Lanes (a)-(d) were *Hind*III digests, (e)-(h) were *Pst*I digests. (a), (b), (e), and (f) were DNA from McLeod subjects, (c), (d), (g), and (h) were from normal subjects. 5µg of DNA was used in each digest. (Right) Lanes (1) and (4) were DNA from a McLeod patient, lanes (2), (3), (5), and (6) from normal subjects. Lanes (1)-(3) were *Hind*III digests, lanes (4)-(6) were *Pst*I digests. The left autoradiograph was hybridised with probe p379-10 and the right with p145-12. After stringent washing in 0.1×SSC at 65°, blots were autoradiographed for 48 hours at -70°, using intensifying screens. *Hind*III digested λ markers were also run and estimated fragment sizes hybridising with the two probes are indicated, bearing in mind that estimates of size around 24 kb are approximate.

Department of Child Health, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. N D Carter, S Jeffery

Department of Histopathology, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF. J E Morgan

Genome Analysis Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX. A P Monaco

Department of Neurology, St George's Hospital Medical School, London SW17 0RE. M S Schwartz

Correspondence to Dr Carter.

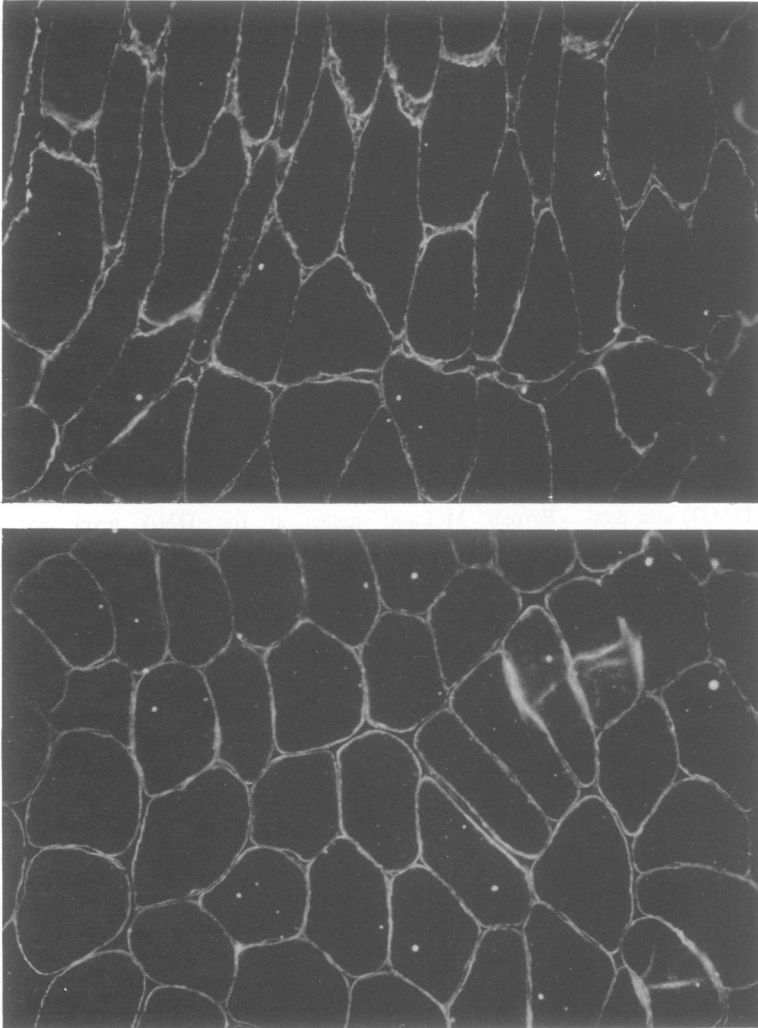


Figure 2 Immunofluorescent localisation of dystrophin in cryostat sections of muscle from the two McLeod subjects.

came to our notice through the South London Transfusion Centre because they had been found to have acanthocytosis during screening of blood donors.² Both cases were extensively studied for possible physiological and pathological abnormalities.²

SOUTHERN BLOTTING

DNA extraction and Southern blotting were carried out using standard protocols.¹ The cloned fragments from the Xp21.1-p21.3 region were used in the analysis. These probes have been used in previous characterisation of deletions in McLeod's syndrome.¹

MUSCLE BIOPSY

Needle biopsies were taken from the right quadriceps

muscles. Cryostat sections (8 μ m) were cut from the frozen muscle biopsies from each patient and were thawed onto gelatin-chrome alum coated slides. Immunofluorescent visualisation of dystrophin was performed as previously described.⁴ PBS containing 5% horse serum was used for blocking of the sections and all reagent dilutions and washes. The primary antibody was a mixture of crude sheep anti-mouse dystrophin antisera directed against cloned dystrophin fusion proteins of 30 and 60 kd molecular weight⁵ and diluted 1/500. The second antibody was a biotinylated donkey anti-sheep (Amersham) diluted 1/400, followed by streptavidin-texas red (Amersham) diluted 1/100. Following the final labelling step, the sections were washed in PBS/horse serum, mounted in gelvatol, and observed with a Zeiss axioscope.

Results

To check for deletions in the two McLeod cases, DNA was digested with *Pst*I and *Hind*III and Southern blots were probed with labelled fragments p145-12 and p379-10. The results are shown in fig 1 and it can be seen that bands of identical migration to the controls were found in the two McLeod phenotypes.

To assess whether the expression of dystrophin was altered in the muscles, dystrophin was localised in the two muscle biopsies (fig 2). The pattern shown is qualitatively identical to control muscles.

Discussion

Subjects with McLeod's syndrome exhibit a mild myopathy which is similar histologically to DMD and produces a raised plasma CK. It is known that the McLeod locus is in the same region as that for dystrophin, since subjects with chromosomal deletions in that area can show both phenotypes. This finding suggested the possibility that there might be some polarity effect on the DMD gene from the McLeod (*Kx*) locus, as it seemed more than chance that two contiguously linked but functionally unrelated loci should produce a similar lesion. Such speculation was shown to be incorrect by the mapping of the McLeod locus some thousands of kilobases from DMD.¹ This leaves open another possible interaction; that there might be an association between the *Kx* glycoprotein and dystrophin which leads to functionally abnormal dystrophin in McLeod's syndrome. Dystrophin is thought to bind tightly with an integral membrane glycoprotein⁶ and it has been suggested that the product of the DMD locus may anchor specific muscle or brain glycoproteins similar to ankyrin and spectrin. Immunostaining of McLeod muscle blocks using antibodies to dystrophin showed no differences compared to control sections from normal subjects. Thus there is evidently no gross deficiency or rearrangement of dystrophin in McLeod patients. However, it does leave open the possibility of structural change in the cytoskeleton, owing to an alteration in the anchoring of dystrophin in the absence of *Kx* glycoprotein, a change that may not be detectable by light microscopy.

Southern blots using probe p145-12, a probe known to detect a deletion in two out of seven McLeod males previously studied,³ showed no gross deletion in either of the two subjects in our study. As both the McLeod patients in this study exhibit the

typical features of the syndrome,² with an absence of *Kx* antigen, this suggests that the large deletion found in the previous study is not the common cause of the phenotype. Probe p379-10, which detected a site adjacent to the previously defined McLeod deletion, also gave a clear single restriction fragment after digestion with *Pst*I and *Hind*III in these two cases. (This probe contains the 3' exon of the CGD cDNA.)

From the above results we conclude that the two unrelated cases of McLeod's syndrome examined do not show a detectable deletion in the same region as the two related males analysed in a previous study.¹ There may be a small deletion or even a point mutation, which our study would not have detected, that could lead to the total loss of the *Kx* glycoprotein. This would not be surprising since some DMD patients show no deletion by Southern blotting, yet produce no dystrophin.⁷ The fact that normal dystrophin expression and distribution occurs in the muscles from the McLeod patients would tend to suggest that the two proteins (*Kx* and dystrophin) do not interact in any substantial way, even though the McLeod and DMD phenotypes do show some common characteristics.

We thank Mrs M Leak and her colleagues from the South London Blood Transfusion Centre. The catalytic advice of Kay Davies is also gratefully acknowledged. Eric Hoffman kindly provided anti-dystrophin serum and Lou Kunkel the probes.

- 1 Bertelson CJ, Pogo AO, Chaudhuri A, *et al.* Localisation of the McLeod locus (XK) within Xp21 by deletion analysis. *Am J Hum Genet* 1988;42:703-11.
- 2 Swash M, Schwartz MS, Carter ND, Heath R, Leak M, Rogers KL. Benign X linked myopathy with acanthocytes (McLeod syndrome): its relationship to X-linked muscular dystrophy. *Brain* 1983;106:717-33.
- 3 Redman CM, Marsh WL, Scarborough A, Johnson CL, Rabin BI, Overbeeke M. Biochemical studies on McLeod phenotype red cells and isolation of *Kx* antigen. *Br J Haematol* 1988;68:131-6.
- 4 Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM. Conversion of mdx myofibres from dystrophin negative to positive by injection of normal myoblasts. *Nature* 1989;337:176-9.
- 5 Hoffman EP, Brown RH, Kunkel LM. Conservation of the Duchenne muscular dystrophy gene in mice and humans. *Science* 1987;238:347-50.
- 6 Campbell KD, Kahl SD. Association of dystrophin and an integral membrane glycoprotein. *Nature* 1989;338:259-62.
- 7 Worton RG, Thompson MW. Genetics of Duchenne muscular dystrophy. *Annu Rev Genet* 1988;22:601-29.