CASE REPORTS

Investigation of a female manifesting Becker muscular dystrophy

I A Glass, L V B Nicholson, E Watkiss, M A Johnson, R G Roberts, S Abbs, S Brittain-Jones, H G Boddie

Abstract

Females manifesting Becker muscular dystrophy (BMD) are even more rarely observed than for the allelic condition Duchenne muscular dystrophy. The male proband has typical BMD with greatly raised CK activity and a myopathic muscle biopsy. His mother experienced walking difficulties from 35 years of age and has a myopathy with marked calf hypertrophy, a raised CK, and a myopathic muscle biopsy. Dystrophin analysis was undertaken on both the proband and his mother. Immunoblotting showed a protein of normal size but of reduced abundance in both. Immunocytochemical analysis in the proband indicated that the majority of the fibres showed weak dystrophin labelling and in his mother both dystrophin positive and dystrophin negative fibres were present. Non-random X inactivation at locus *DXS255*, was observed in DNA isolated from peripheral lymphocytes of the mother. Neither extended multiplex PCR performed on DNA from the proband nor analysis of lymphocyte derived mRNA showed a structural alteration in the dystrophin gene suggesting that an unusual mutation was responsible for BMD in this family.

Becker muscular dystrophy (BMD) has usually been thought of as an uncommon Xp21 muscular dystrophy in comparison to the allelic condition Duchenne muscular dystrophy (DMD) based on studies that predate isolation of the dystrophin gene.¹ However, recent estimates suggest that BMD is more common than previously appreciated.² The disease is characterised in affected males by a slowly progressive proximal myopathy and BMD is a milder condition and has a more variable phenotype than DMD, with an age of onset in BMD that varies from early childhood to the second decade or later.³ Patients with BMD may experience great variation in disability as evidenced by the wide age range at which they become chairbound, but a substantial proportion of subjects never require a wheelchair and have normal longevity. This heterogeneous expression of BMD shows great variability even within families.³

Females clinically manifesting X linked recessive diseases are well documented. This can be as a consequence of Turner's syndrome or as the result of a structural rearrangement of an X chromosome leading to inactivation of the normal X (as in an X;autosome translocation). In addition the manifestation in females who are chromosomally normal is presumed to occur as the result of non-random X inactivation which produces a preponderance of cells with a defective X chromosome active.⁴ All three explanations have been invoked to explain females manifesting DMD.⁵⁻⁸ However, only a few reports exist of females manifesting BMD, ascertained after the diagnosis of BMD in an index male case, in any form other than anecdotes of marginal weakness.⁹

We report a severely affected female with BMD in conjunction with the results of the investigation of the dystrophin gene and related aspects in this family.

Case report

The family whose pedigree is depicted in fig 1 was referred to the genetic counselling service because of concern related to the risk posed to other members of the family, in particular III-2 and IV-1.

The proband had symptoms from early childhood. Upright walking was noted at 14 months of age but there was concern from 4 years of age because of leg pains, inability to run, and difficulty in negotiating stairs. At 6 years, after repeated falls, quadriceps pseudohypertrophy and calf pseudohypertrophy were noted. His serum creatine kinase activity (CK)

Figure 1 Family pedigree.

West Midlands
Regional Genetics
Service, Birmingham
Maternity Hospital,
Birmingham
B15 2TG.
I A Glass
E Watkiss
S Brittain-Jones
Muscular Dystrophy
Group Research
Laboratories,
Newcastle General
Hospital.
L V B Nicholson
M A Johnson
Paediatric Research
Unit, Guy's Hospital,
London.
R G Roberts
S Abbs
Department of
Neurology, South
Staffordshire Royal
Infirmary.
H G Boddie

Correspondence to Dr Glass.

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was greatly raised at 2799 IU/l (upper limit of normal in males 80 IU/l) and an abnormal EMG was recorded from the quadriceps muscle. Muscle histology confirmed a myopathic process. When examined at 23 years of age he was thin with an exaggerated lumbar lordosis and thoracic scoliosis. He toe walked and had a waddling gait using a walking stick for assistance. No facial weakness was evident but generalised wasting and weakness (MRC scales 3/5) of the proximal musculature was noted. Obvious calf pseudohypertrophy was present and Gower's sign was positive. A Vignos rating of 4 was estimated.¹⁰ He was of normal intellect and maintained an independent lifestyle.

His mother, who was not known to be a twin, although asymptomatic at her son's presentation had a raised CK activity (576 IU/l) at this time. She did not develop symptoms until 35 years of age when she complained of slight weakness of her left leg. Examination showed a mild proximal myopathy and asymmetry in the thigh circumferences. The EMG was normal but a muscle biopsy from the quadriceps was myopathic showing increased variation in type I and II fibre size and some fibres with multiple internal nuclei were present. The disease process progressed over the next eight years such that she now cannot climb stairs without assistance and requires a walking stick. She also had distal weakness, being unable to carry out household tasks such as unscrewing of jars. Examination showed a waddling gait with a positive Gower's sign. Facial weakness was not present. Calf pseudohypertrophy was present and generalised wasting and weakness of many muscle groups including the intrinsic muscles of the hand (MRC scale 3 to 4/5) was evident. A Vignos rating of 6 was estimated.¹⁰ There were no known other affected subjects within the family.

LABORATORY INVESTIGATIONS

The raised CK activity of both subjects was confirmed on repeat testing (proband 1700 IU/l and mother, 540 IU/l). The karyotypes were normal, 46,XY and 46,XX respectively.

Using DNA extracted from peripheral lymphocytes of the proband (III-1), a deletion screen of the DMD/BMD gene was performed using an extended multiplex polymerase chain reaction (PCR) designed to amplify exons throughout the dystrophin gene.¹¹ No such deletion was evident. In order to investigate the unlikely possibility of a discrepancy with the PCR result at the 3' end of the gene, cDNA probes Cf56a and Cf56b were hybridised with *Pst*I digested DNA from the proband but no deletion was detected using this approach.

Messenger RNA (mRNA) was extracted from peripheral lymphocytes of the proband as described elsewhere¹² and the coding region of the dystrophin mRNA and promotor mRNA was amplified by reverse transcription and nested PCR with the products visualised on an ethidium stained acrylamide gel.¹² However, no major structural gene alteration was

observed using this methodology. Furthermore a mismatch analysis did not show any abnormality when comparisons to wildtype DNA were made.

Open muscle biopsy on the quadriceps was performed on both the proband and his mother. Immunocytochemical and immunoblotting analyses were carried out in parallel on the specimens obtained, as outlined in Nicholson *et al.*¹³ Two monoclonal antibodies to dystrophin were used for these investigations. One, Dy4/6D3, reacts with an epitope in the central rod domain¹⁴ and the other, Dy8/6C5, recognises an epitope in the last 17 amino acids at the carboxy-terminus.¹⁵ The antibodies are specific for dystrophin and do not react with spectrin, α actinin, or closely related autosomal homologues of dystrophin.¹⁶ A monoclonal antibody which recognises β spectrin in skeletal muscle (RBC2/3D5) was also used in this investigation.

The results of the immunocytochemical labelling with Dy8/6C5 are depicted in figs 2 and 3. Despite only a few residual muscle fibres being present in the biopsy obtained from the proband (fig 2), dystrophin labelling is distinctly reduced confirming an Xp21 muscular dystrophy. In the mother, H&E staining indicates non-specific variability in fibre size and immunolabelling showed a pattern of dystrophin positive, dystrophin negative fibres and partially labelled fibres confirming her as a carrier of an Xp21 muscular dystrophy (fig 3). The proportion of fibres showing a total absence of labelling was 23%, partial labelling was seen on 30%, and continuous labelling on 47% (estimated on a sample of 500 fibres).

The result of an immunoblot labelled with Dy8/6C5 is depicted in the western blot of fig 4. This shows that the proband and his mother have a normal sized dystrophin molecule of approximately 400 kd when compared to that of simultaneously loaded controls. Densitometric analysis was carried out on the dystrophin and myosin bands as outlined elsewhere.¹⁵ The abundance of dystrophin (for the carboxy-terminus) was reduced, estimated to be 68% for the proband and 35% for his mother when allowance was made for the amount of 'muscle protein' extracted from the sample.

In order to study the pattern of X inactivation DNA was extracted from peripheral lymphocytes of the mother and digested with *Msp*I and its isoschizomer *Hpa*II. Both reactions were codigested with *Bam*HI. The Southern blot was hybridised with the DNA probe M27 β which detects the methylation sensitive locus DXS255 at Xp11.22. The results are depicted in fig 5. It is apparent that non-random X inactivation in this tissue is present and that the X chromosome that the proband has inherited is preferentially active in his mother.

Discussion

A rigorous survey to estimate BMD and DMD frequencies by using cDNA probes and dystrophin immunolabelling enabled, particularly

(A)

(B)

(C)

Figure 2 The proband. (A) Muscle shows gross increase in interstitial fibrous connective tissue and fat; only a few residual fibres are present (H&E). (B) Labelling for β spectrin shows that residual muscle fibres preserve near normal reactivity (immunoperoxidase). (C) Muscle section adjacent to that shown in (B). Dystrophin labelling is greatly decreased in the residual muscle fibres (immunoperoxidase).

for sporadic cases, a high proportion of definitive diagnoses to be achieved.² This survey reported the highest incidence (1/18 450 births) and prevalence (2.38/100 000) of BMD yet recorded. The ratio of BMD to DMD is also the highest observed although it was noted that the incidence of DMD had declined in recent years.² The commonest revision of diagnoses were males labelled as having 'limb-girdle' dystrophy or spinal muscular atrophy. After a request for genetic counselling it was decided to reinvestigate this family using newly available dystrophin technology. This was undertaken because of the unusual clinical picture observed in the mother, notably the marked distal involvement. An additional reason to review the diagnosis in this family was provided by the apparent lack of a deletion in the dystrophin gene of the proband, which is in contrast to the usual finding in BMD males who typically have an identifiable deletion in

(A)

(B)

(C)

Figure 3 The mother. (A) Muscle shows increased variation in fibre size but little increase in interstitial fibrous connective tissue (H&E). (B) Labelling for the plasma membrane associated protein β spectrin shows strong reactivity at the surface of all muscle fibres (immunoperoxidase). (C) Muscle section serial to that shown in (B). Labelling for the carboxy-terminus of the dystrophin molecule shows a mixture of dystrophin positive fibres, dystrophin deficient fibres, and partially labelled fibres (immunoperoxidase).

their dystrophin gene.^{17 18} A diagnosis of BMD in this family was confirmed by the dystrophin analysis. Western blot analysis showed a reduced amount of normal sized dystrophin molecules.

In the absence of Turner's syndrome or a structural abnormality of the X chromosome, Lyonisation may be invoked to explain the manifestation of Xp21 muscular dystrophy in heterozygotes. The findings of skewed X inactivation in the peripheral blood of the monozygotic twins discordant for DMD supported the involvement of this mechanism and suggested an influence in determining the twinning process.⁸ Females manifesting X linked conditions would be expected by statistical dictates¹⁹ alone, but good evidence exists for familial aggregation of manifesting DMD heterozygotes⁷ suggesting that either environmental factors are important or that the process of

Figure 4 The upper panel shows a western blot labelled for the carboxy-terminus of dystrophin. The lower panel shows staining of myosin heavy chain in the corresponding gel which is used as a measure of the 'muscle protein' (as opposed to fat and fibrous connective tissue) loaded in each lane. The proband and his mother show bands in the same position as a normal control subject (C), indicating non-deleted dystrophin molecules. Densitometric analysis of the dystrophin and myosin bands indicates that the abundance of dystrophin (for the carboxy-terminus) in both patient samples is reduced (68% for the proband and 35% for his mother).

Figure 5 Autoradiograph after hybridisation of M27 β to MspI/BamHI, and HpaII/BamHI blots using DNA from the proband and his mother. The MspI digest shows that the mother is heterozygous at the DXS255 locus detected by M27 β . In her HpaII lane there is a deficiency of the 10 kb band indicating that the X chromosome represented by this allele is preferentially active in the mother and that X inactivation is non-random.

Lyonisation is genetically influenced. A genetic role is supported by the extreme disparity in phenotypes observed for female haemophilia A within a family studied by Ingerslev *et al.*⁴ In the mother of our proband, there is evidence of non-random X inactivation. Barring recombination between DXS255 and the dystrophin locus her normal X chromosome appears preferentially inactivated in the tissue studied (peripheral lymphocytes). This in

itself may not necessarily indicate an involvement of Lyonisation as skewed X inactivation is demonstrable in both female patients with pyruvate dehydrogenase deficiency and in normal female controls.²⁰ In addition the skewing of X inactivation can show wide variation between different tissues tested within a single subject²⁰ although this is contrary to the consistency observed in levels of glucose-6-phosphate dehydrogenase activity between five different tissue types in heterozygous females.²¹ Ideally, direct analysis of differential methylation at DXS255 in muscle from the proband's mother would be required to address this question further. None the less the extreme variation in dystrophin labelling found in muscle from the proband's mother (including fibres which appeared completely negative) does suggest that the X chromosome bearing the mutation was active in a significant number of myoblasts, thereby accounting for the expression of the BMD phenotype.

This is the first time that such a labelling pattern has been reported for a BMD carrier, although symptomatic DMD carriers have been shown to have 'mosaic' expression of dystrophin at the surface membrane of muscle cells.^{22,23} A 'mosaic' pattern was not evident from the immunolabelling of three asymptomatic BMD carriers (one showing a reduced abundance of dystrophin) studied by Morandi *et al.*²³ although two asymptomatic DMD carriers did show some fibres with negative dystrophin immunolabelling. A BMD heterozygote described by Sunohara *et al.*⁹ with very mild weakness and a myopathic EMG (whose son had a detectable deletion of the dystrophin gene) showed dystrophin bands of both normal and reduced size on western blots. Dystrophin negative fibres were observed in this instance. It appears premature to speculate whether the detection of dystrophin negative fibres in BMD/DMD carriers accurately predicts clinical features as the number of subjects studied is too small.

Even when taking into account the threefold difference in incidence between BMD and DMD² there appears to be a paucity of manifesting BMD heterozygotes compared to DMD heterozygotes (eliminating those with X chromosome structural changes). However, it may be that females manifesting BMD are simply not recognised as the phenotype would be expected to be mild or perhaps be misdiagnosed.²⁴ There remains the possibility that the mother's manifesting state is either the result or the cause of an undetected monozygotic twinning event.¹⁹

The majority of males with DMD/BMD can be identified as having a structural rearrangement in the dystrophin gene,^{17,18,25} but such an event does not appear to have occurred in this family. Thus the defect may either be a point mutation or be the result of undetected alternative splicing of the transcript. The high proportion of dystrophin negative fibres in the mother is similar to that observed in biopsies obtained from manifesting DMD carriers and, interestingly, the level of functional impairment is now more advanced than that of her

son, having progressed rapidly since the onset of symptoms. A recent series of females presenting with a muscular dystrophy in association with a raised CK measurement showed that the majority had an Xp21 muscular dystrophy²⁶ suggesting that a CK estimate is a useful predictor for deciding whether to proceed to dystrophin analysis. This investigation highlights the usefulness of dystrophin protein analysis in securing the correct diagnosis in a female with a muscular dystrophy drawn from a family with a non-deleted index male case.

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