Centronuclear myopathy

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SUMMARY Centronuclear myopathy occurring sporadically in two African female children is reported, with details of clinical history and histological, histochemical, and ultrastructural findings, and a review of 58 previously reported cases. In spite of distinctive histological features, the clinical presentation of this condition is variable, there are different modes of inheritance, and the pathogenesis remains unclear.

Since first described by Spiro *et al.* (1966), many cases of centronuclear myopathy have been added to the literature. It has become evident that although histological features may be distinctive, the clinical presentation is variable and different modes of inheritance may occur. We add two further cases of sporadically occurring centronuclear myopathy. The occurrence of a diffuse myopathy with craniofacial involvement is highly suggestive of the diagnosis and is frequently described in case reports.

Case reports

CASE 1

The patient (CM), a black girl aged 7 years, is the only child of a non-consanguineous marriage. Pregnancy and delivery were normal. In the early neonatal period, transient jaundice was present. A generalised floppiness was noted soon after birth with reportedly normal limb movements. There were no feeding difficulties and bulbar function appeared to be good. Considerable difficulty in lifting the head, especially when in the prone position, was noted after a few months. She was able to sit unaided at the age of 4 months, but was never able to crawl. She walked at the age of 4 years. The patient was first seen at the age of 4 years 9 months because of increasing difficulty with walking. Increasing difficulty had also been experienced in changing from a lying to a sitting position. Her weight was 19 kg and her height 1200 mm. She was moderately obese, with no evidence of fasciculation or myotonia. Mild wasting was evident in the small muscles of the hands, and the ends of

the fingers were tapered (Fig. 1). A marked generalised hypotonia was present. The strength was markedly reduced in the limbs, the upper limbs being slightly weaker than the lower limbs with the proximal muscles being most involved. The neck musculature was diffusely weakened. She was unable to stand from a lying position, could not climb stairs, and walked with a slow waddling gait. Prominent facial weakness resulted in an expressionless appearance. A mild ptosis and proptosis were present. She was unable to raise the eyes, and horizontal upward and downward eye movements were limited to a few degrees. Pupillary reflexes were normal. The masticatory muscles were weakened, and at rest the patient presented an open-mouthed appearance (Fig. 2). The tongue and pharyngeal muscles were normal. The trunk musculature was similarly involved, resulting in an exaggerated lumbar lordosis with protuberant abdomen. Respiration was mainly abdominal. The tendon reflexes were absent and plantar responses flexor. Sensation was normal. Over the vertex of the head there was a patch of white hair.

Fig. 1 Tapering of distal ends of fingers. Case 1 (CM).

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Fig. 2 White patch of hair over vertex. Ptosis and open-mouthed appearance are evident. Case 1.

Electromyography of the biceps, triceps, quadriceps, and abductor pollicis brevis muscles showed low voltage polyphasic motor unit potentials which were interpreted as myopathic. No fibrillation potentials were seen. Motor nerve conduction velocity in the ulnar nerve was 51.5 m/s and 45.9 m/s in the peroneal nerve.

The serum creatinine phosphokinase (CPK) level was minimally raised at 32.2 iu (normal 0–30) and the serum aldolase was 41.0 iu (5–31). A biopsy of the rectus femoris muscle was performed.

The patient was seen again at the age of 6 years 10 months. Her weight was 26 kg. Slow deterioration of muscle strength had occurred, and there was increasing walking difficulty. Examination revealed a similar pattern of muscle weakness as had been noted on the first visit. Her mental state and general physical examination was considered to be within normal limits. Repeat serum muscle enzyme estimations showed normal values. The results of the full blood count, serum calcium, potassium, chloride, urea, sodium, and thyroid functions were normal. A second muscle biopsy was obtained from the biceps muscle.

Clinical examination of the mother revealed no

abnormality. There was no family history of neuromuscular disorder. The father was not available for examination.

CASE 2

A black girl (QM) aged 14 years was born prematurely at 7 months. The mother did not experience fetal movements during pregnancy. At birth the child was floppy and apnoeic, and required resuscitation. Although she sat, she was never able to crawl, stand, or walk. There were no feeding problems or dysphagia. Throughout childhood there had been recurrent respiratory infections requiring frequent hospitalisation. In early childhood she developed flexion contractures of the lower limbs. Her mental state was normal but emotionally she was apathetic. There was an almost total ophthalmoplegia and bilateral mild ptosis. Pupillary function was intact and the fundi normal. The face was thin and elongated, and the skin contained numerous lentigines. Marked weakness of the face, masticatory, and anterior and posterior neck muscles was present. The tongue and pharyngeal muscles appeared normal. There was marked weakness and wasting of the proximal and distal muscles of the limbs. Flexion

Fig. 3 Elongated face, generalised wasting and contractures of lower limbs. Case 2 (QM).

contractures of the hips and knees and of the extensors of the wrists were present (Fig. 3), and there was generalised hypotonia with hyperextensibility at the elbow. There was no myotonia or fasciculation. Tendon reflexes were absent, and the plantar responses were flexor. Sensation and co-ordinatory function were normal. The blood pressure, pulse, and general examination revealed no abnormality.

There is no known family history of a neuromuscular disorder. One sibling, a stepbrother, is clinically normal. The mother showed no abnormality on detailed clinical examination, and her creatine phosphokinase level in the serum was normal. The father was not available for investigation but is apparently normal.

Normal biochemical findings included full blood count, haemoglobin, blood urea, electrolytes, calcium, and phosphorus. A chest radiograph revealed inactive tuberculosis.

Electromyography of the biceps muscle revealed scattered scanty, fibrillation potentials. The volitional pattern showed small (200–400 μ V) short duration, polyphasic motor unit potentials. Motor conduction velocity in the left median nerve was 70 m/s.

A biopsy of the biceps muscle was performed on the patient and her mother.

Methods

Muscle obtained for routine histology was fixed in 10% formalin, embedded in paraffin, and sections stained with haematoxylin and eosin, phosphotungstic acid haematoxylin (PAH), periodic acid Schiff (PAS), and Gomori's trichrome. Muscle specimens frozen in isopentane cooled in liquid nitrogen were submitted for histochemistry as described by Dubowitz and Brooke (1973). The following reactions were performed: routine adenosine tri-phosphatase (ATPase) at pH 9.4, modified ATPase after pre-incubation at pH 4.6 and 4.3, phosphorylase and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) reactions.

Specimens for electron microscopy were fixed in 2.5% glutaraldehyde in cacodylate buffer and post-fixed in osmium tetroxide. A histogram of muscle fibre diameter was constructed by measuring the mean diameter of 559 fibres (Fig. 4).

Results

LIGHT MICROSCOPY

Case 1

There was a mild to moderate increase in the amount of interstitial fat, but the most prominent feature was the presence of many very small fibres with an average diameter of 10.4 μ m (range 5–50 μ m). Many of these

Fig. 4 Histogram of muscle fibre diameter. Case 1.

fibres (25%) had central nuclei, and in most of them no subsarcolemmal nuclei were observed. A clear space devoid of myofibrils was evident surrounding many of the central nuclei. On longitudinal sections the central nuclei could be seen in over 80% of fibres, and were often arranged in long chains, and clear areas around the nuclei were prominent (Fig. 5). On transverse sections some fibres showed central clear areas which were unrelated to a nucleus, but PAS positive material within these areas was not seen. Occasional large hypertrophied fibres were seen with a diameter of 40–50 μ m. Clusters of centrally situated nuclei were present in a number of these large fibres. Occasional abnormal architecture was noted on sections stained with PAH, and these consisted of loss or a disorderly arrangement of the normal striatal pattern. Muscle spindles were normal in appearance, and the small size of many of the fibres was well demonstrated in comparison with spindles (Fig. 6).

Case 2

Marked atrophy of fibres was present. The average diameter was 14.2 μ m with a range of 2–70 μ m and mode at 8 μ m. Most fibres had a diameter of less than

Fig. 5 Central nuclei, occurring in small groups or long chains. Case 1. H and E, original magnification $\times 1350$.

Fig. 6 Small size of fibres seen in relation to muscle spindle. Case 1. H and E, original magnification $\times 340$.

20 μ m. Central nuclei were present in more than 90% of fibres on longitudinal section and in 40% of fibres on cross-section. Fibres contained from one to six internal nuclei, and subsarcolemmal nuclei were commonly associated, especially in longitudinal sections. Many of the larger fibres showed a circular or transverse internal splitting with nuclei aligning themselves along the split (Fig. 7). Central clear areas were present and occasional fibres demonstrated a perinuclear halo. There was a moderate endomysial and perimysial fibrosis and fatty infiltration.

HISTOCHEMISTRY

Case 1

There was poor differentiation into fibre type in oxidative enzyme preparations. In the central areas of

some fibres there was evidence of increased activity, while other fibres showed decreased activity. Scattered fibres stained very densely and in some there was a rim of intense staining, while in other fibres there was marked disorganisation of the normal architecture (Fig. 8).

With adenosine triphosphatase there was poor differentiation of fibre type at pH of 9.4 and 4.6, but at a pH of 4.3 some of the larger fibres showed inhibition of the reaction, as well as occasional smaller fibres (Fig. 9). It was thought that most fibres were of type 1, or undifferentiated.

Case 2

With the ATPase reaction, most fibres stained uniformly and were considered to be type 1 fibres.

Fig. 7 *Fibres showing multiple internal nuclei and internal splitting. Case 2. H and E, original magnification $\times 420$.*

Fig. 8 *Variable reaction, some fibres showing peripheral ring enhancement, or irregular or diffuse dense reaction. Case 1. NADH-TR, original magnification $\times 1100$.*

Fig. 9 *Occasional larger fibres show inhibition of reaction. Case 1. APTase pH 4.3, original magnification $\times 225$.*

Occasional fibres, both large and small, stained more darkly like type 2 fibres. Many fibres showed a lack of reaction in the central areas; occasional fibres showed an increased staining pattern in the centre. The NADH-TR reaction showed a poor differentiation of fibre types. Larger fibres tended to stain more lightly. The cytoarchitectural pattern was variable, demonstrating either increased or decreased central staining or a uniform pattern.

LM (mother of case 2)

Biopsy samples of the biceps muscle revealed no abnormality. Histochemical examination was normal.

ELECTRONMICROSCOPY

The sarcolemma and basement membrane were normal in appearance in case 1. The central location of many nuclei within the muscle fibre was confirmed while other nuclei were seen in the subsarcolemmal situation. The outline of some nuclei showed irregular indentations but others retained the normal regular pattern. A prominent nucleolus was seen in some of the nuclei. The chromatin pattern was normal. The clear space which had been seen surrounding some of the nuclei on light microscopy could not be demonstrated at the ultramicroscopic level, and in several instances myofibrils were seen close to the nuclear membrane. Occasional nuclei were surrounded by amorphous material and glycogen granules. Disruption of the myofibrils was prominent with areas showing complete disorientation, and such changes were not only seen in association with central nuclei (Fig. 10). Myofibrillar breakdown was often confined to the more central portion of the fibre. The Z

band was abnormal, and smearing and distortion were present in some situations.

There was occasional reduplication of the sarcolemmal triad (Fig. 11). In other areas of myofibrillar disruption, interesting focal changes were noted in the myofilaments (Fig. 12). Focal thickening of adjacent myofilaments resulted in laminated structures superficially resembling replicated sarcoplasmic triads. Such changes were associated with gross Z band disruption. The mitochondria were normal and no myelin figures were seen.

The ultrastructural findings in case 2 were similar to those in case 1.

Discussion

GENETICS

Of the 58 cases described in the literature the mode of inheritance has been X-linked in two pedigrees (van Wijngaarden *et al.*, 1969; Barth *et al.*, 1975), and autosomal dominant in one family over five generations (McLeod *et al.*, 1972). Sporadic cases are described in 11 further reports although in the original description by Spiro *et al.* (1966), there was distant consanguinity. In the reports by Sher *et al.* (1967), Munsat *et al.* (1969), Karpati *et al.* (1970), and Schochet *et al.* (1972) there was involvement of the mother and daughter, although the mother was usually subclinically affected. In the description by Bradley *et al.* (1970), two male siblings were affected. The remarkable report by Bethlem *et al.* (1969) describes a sibling with peroneal muscular atrophy and the father with a subclinical myopathy, not specifically characterised as centronuclear. If one excludes the two families with X-linked recessive

Fig. 10 Disruption of myofibrils is marked and in this section is not in association with central nuclei. Case 1. EM, original magnification $\times 4770$.

Fig. 11 Replication of sarcoplasmic reticulum. Case 1. EM, original magnification $\times 47\,700$.

Fig. 12 Disorganised area with focal laminated thickening of myofilaments. Z band disruption is prominent. Case 1. EM, original magnification $\times 29\,800$.

inheritance there is a more or less equal sex incidence (23 females, 18 males). Thus at least two modes of inheritance are apparent.

CLINICAL FEATURES

Both of our cases showed obvious extraocular muscle involvement with ptosis, marked facial diplegia, and muscle weakness. The tongue was normal. These features are not invariable as judged by the cases reported in the literature. Ptosis was noted in 15 cases, and extraocular and bulbar facial involvement in 12. It is possible that those patients who died in infancy might have demonstrated oculobulbar involvement at a later date. Limb and trunk involvement showed

varying patterns in which weakness could be symmetrical, or maximally proximal or distal. Hypotonia and atrophy appear to be common features. The tendon reflexes are usually absent. The most common associated abnormality described is kyphoscoliosis, but equinovarus deformity of the foot, pectus excavatum, facial asymmetry, hypertelorism, and café-au-lait spots also occur. One patient described by Headington *et al.* (1975) had sensory neuropathy.

The mode of presentation and age of onset has also been variable. Both families with X-linked pattern of inheritance and some of the sporadic cases showed marked abnormalities at birth. Asphyxia, cyanosis, floppiness, and bulbar and respiratory difficulties

were the usual initial clinical features. This may be specific for the X-linked recessive variety. Another common method of presentation is with delayed motor development in infancy or slowly progressive limb girdle weakness in childhood. However, later onset in the second or third decade, as exemplified by the family reported by McLeod *et al.* (1972), or even presentation in the elderly (Harriman and Haleem, 1972), may occur.

Serum muscle enzymes show a variable pattern but are usually within the normal range, and when elevated the rise is usually moderate. Electromyography has, in most cases examined, shown a myopathic pattern, occasionally associated with fibrillation potentials.

HISTOPATHOLOGICAL CHANGES

Differentiation of fibres into types 1 and 2 is described in some reports (Brooke and Williamson, 1969), while in other reports poor differentiation made typing difficult (van Wijngaarden *et al.*, 1969; Harriman and Haleem, 1972; Headington *et al.*, 1975). In one case described by Headington *et al.* there was a predominance of type 2 fibres. In our own two cases there was a marked predominance of type 1 fibres as judged by the ATPase reaction performed with preincubation at pH 9.4. However, it should be noted that no clear-cut reversal of the reaction was obtained on preincubation at pH 4.3. Where type 1 and type 2 fibres can be distinguished, the type 1 fibres have generally been found to be of a smaller average diameter (Engel *et al.*, 1968; Bethlem *et al.*, 1969; McLeod *et al.*, 1972; Headington *et al.*, 1975).

The oxidative stains usually show one of three patterns. There may be uniform staining or an increase or decrease of the reaction in the central area. In our first case, although this pattern was seen, many fibres also showed a more general disorganisation of the cytoarchitectural pattern. In histopathological studies the most striking finding is the presence of central nucleation, in which there may be from one to several nuclei per fibre. Reports indicate that the percentage of fibres showing central nuclei on examination of cross-sections may be quite variable. It is likely that fibres showing a clear central area will contain a central nucleus at a higher or lower level. Careful examination of longitudinal sections usually reveals that most fibres contain central nuclei, often arranged in chains. Internal splitting with nuclei aligned along the split is a not uncommon appearance, especially in larger fibres. Correlation with ultrastructural studies in our two cases would suggest that this is probably an artefact of fixation as myofibrils and other constituents were not found to be separated from the nuclei. On longitudinal sections chains of nuclei are characteristically seen. Literature reports of fibre size are

very variable, but the presence of many small fibres is usual.

The ultrastructural findings have also shown some variability. Central nuclei may show deep indentations (Vital *et al.*, 1970). A disordered architecture in the central region containing glycogen, mitochondria, membrane-bound material, lipofuscin, and sometimes myelin figures was found by many investigators. In our two cases these features were present, but no myelin figures were seen. We also noted that these destructive changes may be present in the absence of central nucleation. A dilated or reduplicated sarcoplasmic reticulum was a prominent feature, especially in our case 1. This has been observed by others (Harriman and Haleem, 1972; McLeod *et al.*, 1972). In areas of disorganised myofibrils, Z band abnormalities such as streaming may be seen. Headington *et al.* (1975) noted a decreased diameter of axial compared to subsarcolemmal myofibrils, while Bethlem *et al.* (1968), and Badurska *et al.* (1969) found no degenerative changes.

The pathogenesis of this condition is unclear. An arrest of development in the fetal stage has not been favoured since the original suggestion by Spiro *et al.* (1966). In many cases it seems reasonable to assume that the condition becomes established in utero since a history of absent or decreased fetal movements may be obtained, and physical signs may be present at birth. Equally, however, many cases become manifest later in life or remain subclinical.

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