

Motor Neuron Degeneration in a 20-Week Male Fetus: Spinal Muscular Atrophy Type 0

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ABSTRACT: *Background:* Neuropathological changes in degenerating motor neurons are well documented in the term neonate with spinal muscular atrophy, but not at midgestation. *Methods:* Postmortem neuropathological examination was performed in a 20-week male fetus with a hypoplastic left cardiac anomaly. *Results:* Selective degeneration of spinal and hypoglossal motor neurons was an incidental finding. Degenerating motor neurons were not immunoreactive with neuronal nuclear antigen (NeuN) or neuron-specific enolase (NSE), as were the normal motor neurons. Synaptophysin reactivity was reduced around the soma of degenerating normal motor neurons. Ubiquitin and tau were expressed in degenerating motor neurons. Gliosis, inflammation and microglial activation were lacking in the ventral horns of the spinal cord. Laryngeal striated muscle was unaltered for age. No cerebral malformations or hypoxic-ischaemic changes were found. *Conclusion:* This case represents an early motor neuronal degeneration and corresponds to the recently described "type 0" spinal muscular atrophy. Lack of contractures is attributed to the early fetal age, since most muscular growth occurs in the second half of gestation.

RÉSUMÉ: *Dégénérescence du neurone moteur chez un fœtus de sexe masculin de 20 semaines : atrophie musculaire progressive de type 0.* *Introduction:* Les alterations des neurones moteurs dégénératifs dans le nouveau-né à terme sont bien documentés, mais pas en la gestation moyenne. *Méthodes:* On a réalisé l'examen neuropathologique postmortem dans un foetus masculin de 20 semaines qui avait le syndrome d'hypoplasie du coeur gauche. *Résultats:* Nous avons trouvé une dégénération selective des neurones moteurs comme trouvaille fortuite. Les neurones moteurs dégénératifs ne sont pas immunoréactifs avec l'antigène neuronale nucléaire (NeuN) ou l'énolase spécifique aux neurones, bien que les neurones moteurs bien conservés montrant ces reactivités. La synaptophysine est moins réactif au cours des neurones dégénératifs que dans les neurones moteurs normaux. L'expression d'ubiquitine et de tau aussi se trouve. Il n'y a pas de gliose, d'inflammation ou d'activation microgliale. Le muscle estrié paralaryngéal est normale à cette âge. Les malformations cérébrales et les alterations hypoxiques-ischémiques sont absents. *Conclusion:* On propose que ce cas représente le changement pathologique plus tôt dans les neurones moteurs de l'atrophie spinale musculaire et correspond au «type 0» récemment décrit. L'absence de contractures s'attribue à l'âge jeune foetaux puisque le croisement plus rapide du muscle a lieu dans la seconde moitié de gestation.

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Spinal muscular atrophy (SMA) is traditionally classified as three clinical forms based upon age of presentation, severity of weakness, rate of progression and life expectancy. Type I (infantile SMA; Werdnig-Hoffmann disease) is traditionally considered the most severe form, with manifestations already evident at birth; 75% of cases die by two years of age, most within the first few months. Type II (intermediate SMA) also presents early, though not always in the neonatal period, has a slower progression and longer longevity. Type III (juvenile or late SMA; Kugelberg-Welander disease) does not exhibit weakness early in infancy and has the slowest progression, patients often living into adult life and even experiencing a

clinical plateau without further progression.¹⁻⁴ All three types are associated with mutation or excessive repeats in the SMN1 gene on chromosome 5, though other forms of SMA exist that do not

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involve this gene. Approximately ten percent of cases of type I SMA are born with congenital contractures.⁵ An even more severe congenital SMA has recently been described as “type 0”, characterised clinically by arthrogryposis multiplex congenital, severe weakness including dysphagia at birth, and death in early infancy.⁶⁻⁹ Motor neuron degeneration demonstrated neuropathologically in a 20-week human fetus is poorly documented, particularly as an unexpected, incidental finding at postmortem examination. We propose that this fetus represents SMA type 0, with absence of contractures attributed to the early age, before the major growth of fetal muscle.

CASE REPORT

This male fetus was born at 20 2/7 weeks gestation to 27-year-old gr 2 para 1 ab 0 mother after an uneventful pregnancy including lack of maternal infections and drug or alcohol abuse. The pregnancy was terminated because of prenatal echocardiographic diagnosis of hypoplastic left heart syndrome with only two cardiac chambers, right atrium and ventricle identified; the left ventricular outflow tract appeared absent and the right was large; the ascending aorta was severely hypoplastic. Cytogenetics from amniocentesis revealed a normal 46XY karyotype. The cardiovascular findings were confirmed at autopsy, and no other external or visceral anomalies were found. The placenta was normal, consistent with a second trimester gestation. Weight of the fetus was 275.0g; the crown-rump length was 16.0cm and crown-heel length was 23.6cm. Family history was negative for neurological and neuromuscular diseases.

Neuropathological examination of the brain revealed no malformations. The unfixed weight was 45.5g (normal mean for age 50g). The spinal cord was macroscopically normal, with the expected enlargements in the cervical and lumbar regions and normal nerve roots and cauda equina. Microscopic examination of the brain showed no evidence of hypoxic/ischaemic neuronal

alterations. Maturation corresponded to midgestational age, including the immunocytochemical studies as described below. Abnormalities were limited to motor neurons of the ventral horns of the spinal cord and hypoglossal nuclei of the medulla oblongata; oculomotor nuclei were spared.

At cervical, thoracic, lumbar and sacral levels, the general architecture of the spinal cord was normal and the ependymal-lined central canal was patent and of normal size, and the dorsal median septum was well formed in the dorsal midline. Blood vessels, both intra- and extra-medullary, appeared normal. Most motor neurons appeared normal in morphology, including uniform cytoplasmic distribution of Nissl granules, but scattered motor neurons had central chromatolysis. About 10 to 20 percent of motor neurons at all levels exhibited cytoplasmic shrinkage and loss of Nissl substance, associated with either dark, dense nuclei or very pale nuclei and indistinct chromatin with haematoxylin-eosin stain (Figure 1). Similar, but milder, changes were observed in the hypoglossal nuclei of the medulla oblongata and in the trigeminal motor nuclei of the pons, but the oculomotor nuclei of the midbrain had uniformly well preserved motor neurons. Neuronal alterations were not demonstrated elsewhere in the central nervous system. No inflammatory cells were demonstrated in the ventral horns or elsewhere, and no abnormal inclusions were found. Acridine orange confirmed a loss of ribosomal RNA fluorescence from altered motor neurons, and normal bright orange-red fluorescence in the cytoplasm of preserved motor neurons and other neurons, similar to findings previously reported.¹⁰

Immunocytochemical markers

Motor neurons with normal morphology showed strong immunoreactivity in both nuclei and cytoplasm with neuronal nuclear antigen (NeuN), but the pyknotic cells were nonreactive (Figure 2). Neuron specific enolase (NSE) similarly showed strong reactivity in preserved motor neurons and loss of

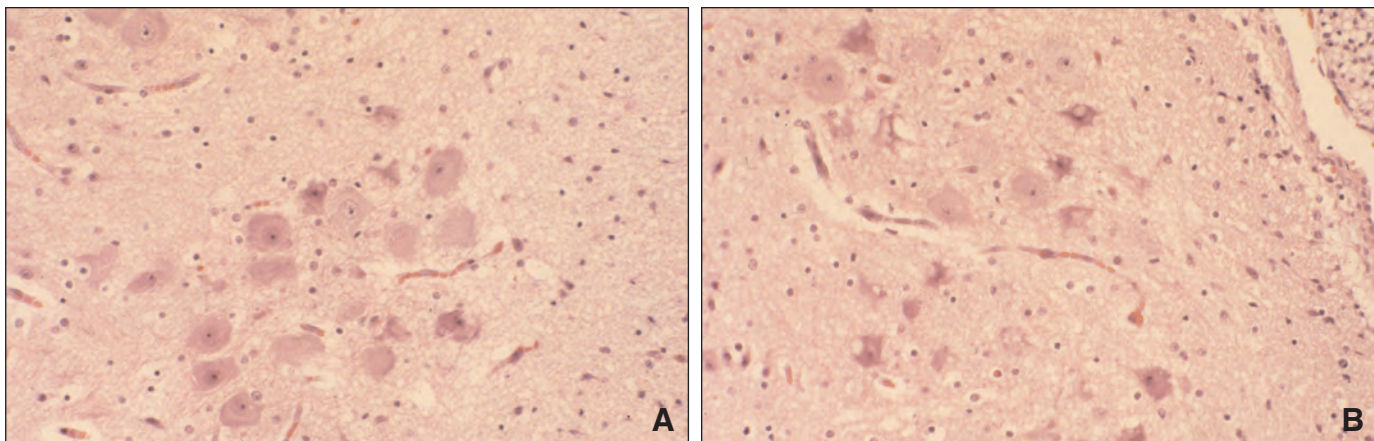


Figure 1: Motor neurons in the ventral horns of the (A) cervical and (B) lumbar regions of spinal cord. Most motor neurons have normal morphology, but many cells scattered between normal ones appear pyknotic with shrunken cytoplasm and dense nuclei. No inflammatory cells are seen. Microglial activation also was negative with CD-68 antibody (not shown). Haematoxylin-eosin. X400.

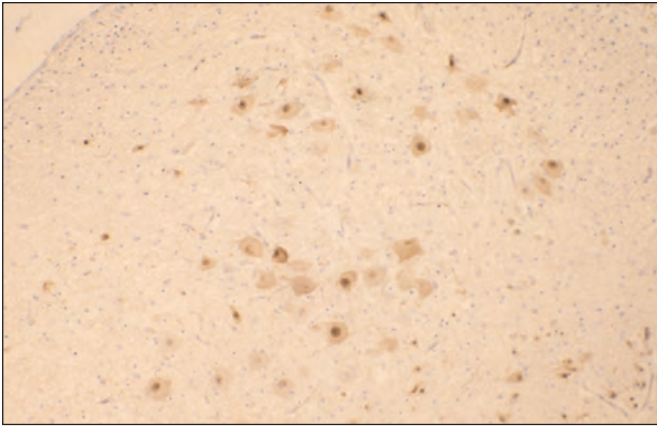


Figure 2: The normal-appearing motor neurons express neuronal nuclear antigen (NeuN), but the shrunken, degenerating motor neurons are nonreactive. X400.

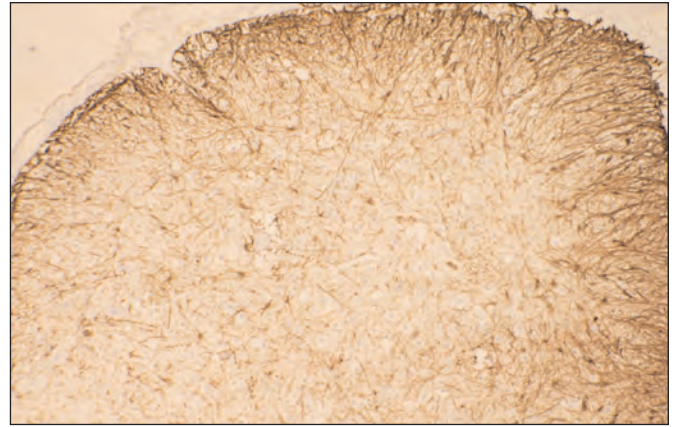


Figure 4: No gliosis is seen in ventral horns. Normal radial astrocytic processes are seen in peripheral white matter. Glial fibrillary acidic protein, monoclonal antibody (GFAP). X250.

reactivity from altered motor neurons, but some normal-appearing motor neurons also failed to be marked by NSE (Figure 3). Synaptophysin reactivity was normally strong throughout the dorsal and ventral horns of the spinal cord, but the strong synaptic vesicle reactivity surrounding preserved motor neurons was sparser or absent around altered motor neurons. Neither vimentin nor glial fibrillary acidic protein (GFAP) demonstrated proliferation of glial cells or reactivity within normal or abnormal motor neurons (Figure 4). CD-68 showed scattered microglial cells in the ventral horns, particularly at the periphery, and rare small clusters as early microglial nodules. No gliosis of proximal ventral or dorsal nerve roots was demonstrated. Tau antibody was reactive in a few of the degenerating motor neurons. Ubiquitin was reactive in the cytoplasm as well as in some nuclei of both degenerating and

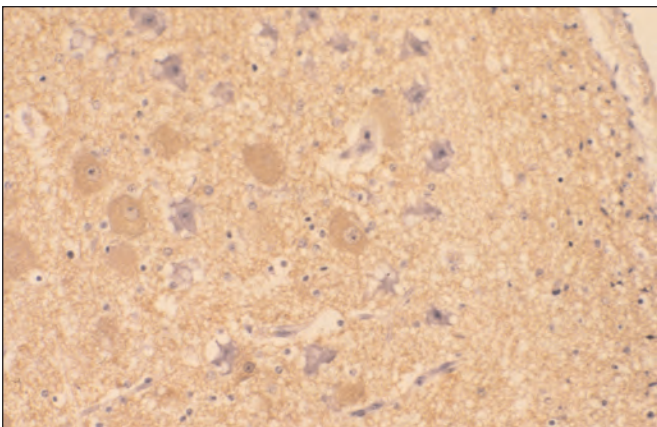


Figure 3: Neuron-specific enolase (NSE) is expressed in most histologically normal motor neurons, but not in degenerating cells. X400.

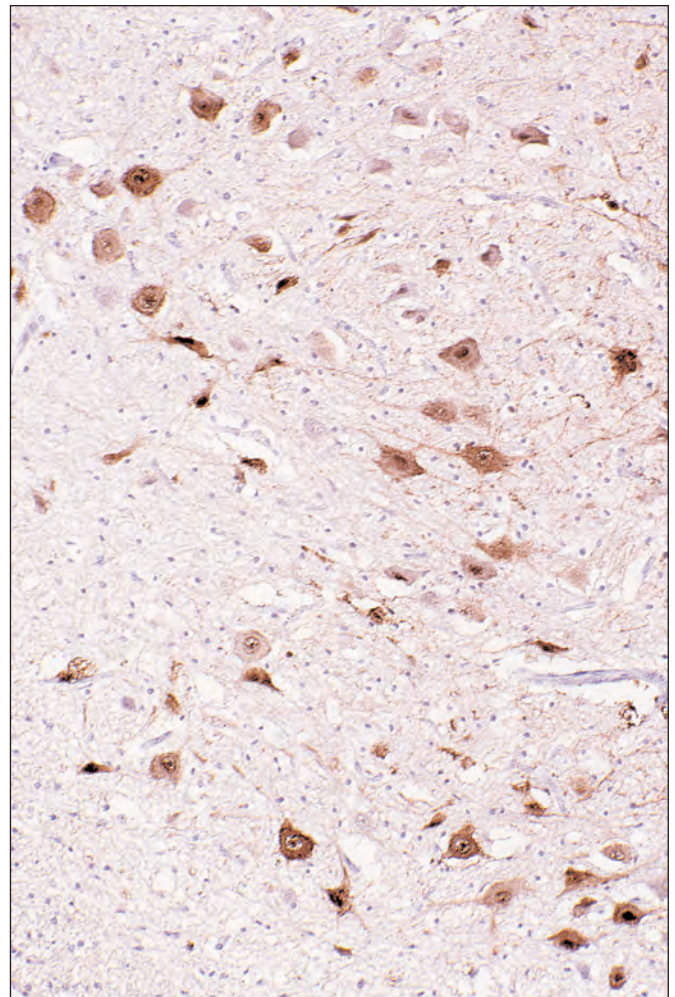


Figure 5: Neuronal nuclear antigen (NeuN) reactivity is demonstrated in all lumbar ventral horn spinal motor neurons in an age-matched 20-week control fetus with a normal spinal cord. X400.

preserved motor neurons. Immunoreactivity for bcl-2 was negative in motor neurons. Three control spinal cords were available from age-matched fetuses at midgestation: degenerating motor neurons were not demonstrated and all motor neurons expressed normal NeuN (Figure 5), NSE and synaptophysin reactivities.

Striated muscle

Because motor neuron degeneration was not suspected prenatally or at the time of autopsy, no muscle of the extremities was sampled, though a piece of diaphragm was taken. Paraffin sections of the larynx included regional striated muscle. Both paralaryngeal muscle and diaphragm showed pathological changes. About ten percent of myofibres were centronuclear and no myofibre degeneration or cytoarchitectural alterations were seen. Acridine orange showed no abnormal RNA fluorescence to indicate regeneration or fibres arrested in the myotubular stage. Myosin and actin markers were not performed because histochemical differentiation of fibre types is not expected at midgestation.

Genetic studies

Molecular analysis of DNA extracted from frozen thymic tissue did not detect a homozygous deletion of exons 7 and 8 of the SMN gene. In our case, because the diagnosis of SMA was not suspected until the spinal cord was examined microscopically, neither blood sample nor frozen muscle tissue was taken for genetic confirmation. Frozen thymic tissue was available and was tested for the SMN1 gene with normal results. The asymptomatic parents were not tested for the SMN gene.

DISCUSSION

The degenerative changes in motor neurons and absence of these changes in age-matched controls was accepted as convincing evidence of a spinal muscular atrophy, though an SMN genetic defect could not be confirmed. We regard this as a form of the recently described type 0 and attribute the lack of contractures to the early gestational age, before the major growth of fetal muscle has occurred.

Motor neurons differentiate early in ontogenesis, induced by Sonic hedgehog homologue (SHH) from the notochord and floor plate ependyma, and innervate their muscular targets at 9-11 weeks gestation; they are mature well before midgestation.¹¹ Physiological apoptosis of motor neurons occurs mainly before that time, and is completed shortly thereafter. By midgestation, the presence of degenerating motor neurons cannot be attributed to normal programmed cell death, and indeed none of our age-matched control spinal cords exhibited motor neuron degeneration.

The pathogenesis of SMA involves persistence into late fetal and postnatal life of an early developmental process of apoptosis of spinal motor neuroblasts that are generated in excess of the number needed for mature motor units. The physiological process of apoptosis becomes pathological if not arrested at a programmed time in ontogenesis. This mechanism was predicted¹² and subsequently proved genetically in two laboratories.^{13,14} The SMN gene is expressed very early in gestation, even in neuroepithelial cells, and is expressed in all

neurons, not just motor neurons, with a progressive shift in subcellular localisation of the SMN protein from mainly nuclear, then cytoplasmic and finally axoplasmic sites during maturation.¹⁵ A specific role for SMN may involve axonal growth, sprouting of collaterals and terminal ramifications.¹⁵ A minority of cases of SMA does not exhibit this SMN mutation, but are clinically very similar, hence SMA may be due to other genes not yet known.

In the case here presented, degenerating motor neurons were seen histologically at all levels of the spinal cord and in the hypoglossal nuclei. This finding was confirmed by a lack of immunoreactivity with the neuronal markers NeuN and NSE in these degenerating cells, with normal expression of these proteins in adjacent normal motor neurons. Ubiquitin is a marker of motor neuron degeneration in adult,¹⁶ and was positive in this case also, more strongly expressed in the degenerating than in adjacent normal motor neurons. Tau protein also was expressed in some degenerating motor neurons, though fewer than ubiquitin-positive cells. The lack of generalised microglial activation and only rare microglial nodules in the ventral horns of the spinal cord, shown by CD-68 antibody, is likely a function of the early age of the fetus and inability to mobilise microglia as readily as more mature neural tissue can do. Gliosis also was absent by GFAP, but reactive astrocytic proliferation is rare at midgestation.

The most frequent forms of SMA are due to deletions in exons 7 and 8 of the telomeric survival motor neuron (SMN) gene at locus 5q13; this gene normally has two copies, a telomeric SMN1 and a centromeric SMN2 and transmitted as an autosomal recessive trait.¹⁻⁴ Other SMAs also are known that do not involve this gene or its associated neuronal apoptosis inhibition protein gene (NAIP). Examples include the motor neuron degeneration in pontocerebellar hypoplasia type 1,¹⁷⁻¹⁹ and that occurring in some cases of the Pena-Shokier phenotype or fetal akinesia sequence²⁰ and the Marden-Walker syndrome.²¹ Our fetus did not have any of these disorders, nor did he have Tay-Sachs disease, Pompe disease or other metabolic storage diseases that might involve motor neurons because the characteristic abnormalities in brain, heart, liver and other organs were lacking. Sporadic cases of nonsyndromic SMA without SMN gene mutations also are described: an autosomal dominant mild SMA, and an X-linked recessive form at Xq11-q12, are documented. Another form of infantile progressive SMA with respiratory distress (SMARD1) because of early diaphragmatic involvement, by contrast with the relative diaphragmatic sparing in the more frequent 5q13 SMA, is at the 11q13-q21 locus and appears to be transmitted as an autosomal recessive trait.^{1-4,22,23} Sequence analysis of the IGHMBP2 gene reveals a novel frameshift mutation in exons 12 and 13, resulting in substitution of isoleucine for valine.²³ The infant with type 0 SMA reported by Kizilates et al⁸ may have had the X-linked recessive disease, not related to the SMN1 gene. He had polyhydramnios, lacking in our case, and died at 13 days of age from respiratory failure. The male infant reported by Nadeau et al. showed a homozygous deletion in exon 7 of the SMN1 gene.⁹

Our case had a complex congenital cardiac malformation. The association of such cardiac lesions with SMA is rare, but has been reported in neonates with mutations of the SMN gene.^{1,24-26} Congenital heart disease was once even an exclusion criterion in

the diagnosis of SMA.²⁷ Reports of neonates and fetuses dying with congenital cardiac lesions, by contrast, do not describe abnormalities of ventral horn neurons,^{28,29} and our own experience with autopsies of fetuses and neonates with congenital heart disease also does not reveal motor neuron disease. We conclude that this association is coincidental and that the SMN gene or other genes causing SMA are not also the genetic basis for the cardiac malformation. The selectivity of the involvement of spinal and hypoglossal motor neurons without other changes in neurons of the brain or spinal cord to suggest hypoxic/ischaemic or metabolic encephalopathy indicates that this motor neuron degeneration is not merely one component of systemic or generalised CNS disease.

At midgestation, the age of our fetus, striated muscle is beyond the myotubular stage of development (8-15 weeks) and has not yet begun the stage of histochemical differentiation of fibre type (20-28 weeks).¹¹ It is not surprising, therefore, that the muscle showed a paucity of histopathological changes in this 20-week fetus. The characteristic features of infantile SMA in late fetal life and the postnatal period, groups of giant type I myofibres and fascicles of atrophic, mixed but predominantly type II fibres, would not be evident at midgestation.^{3,11,30} Cycles of denervation and reinnervation also would not have had enough time to show changes in the muscle tissue. Infants with type I SMA have an excessive number of centronuclear fibres, many of which exhibit additional ultrastructural characteristics of arrested true myotubes.^{31,32} In one of the cases reported as type 0 SMA, histopathological examination of muscle also appeared as a centronuclear myopathy and myotubularin was deficient as in X-linked recessive myotubular myopathy.⁹ The diaphragm was normal in our case, but is selectively spared in SMA despite severe involvement of intercostal muscles.¹ The involvement of paralaryngeal muscle in this disease is poorly documented, especially in the fetus and neonate, and was the only other striated muscle available for histopathological examination in our case. Well preserved diaphragmatic muscle in our case render it unlikely to be the recently recognised form of SMA with respiratory distress (SMARD1, see above), but whether the diaphragm is already involved at midgestation in these affected infants is yet unknown. In the common form of Werdnig-Hoffmann disease, the diaphragm is selectively spared, together with extraocular and sphincter muscles.

Clinically, the previous reports of type 0 SMA with arthrogryposis multiplex congenital were born much later in gestation.⁶⁻⁹ All had pathological examination of muscle. Kizilates et al⁸ stated that the spinal cord in their case showed loss and degeneration of motor neurons, but no special neuropathological studies were reported and no other details of the CNS were given. In the case of Nadeau et al,⁹ the infant was still alive at the time of publication. At least some of the 10% of infants with infantile SMA who have congenital contractures⁵ might today be reclassified as type 0. The few recent neonatal cases described as type 0 with phenotype/genotype correlations lacked neuropathological confirmation that we here demonstrate in a fetus. We attribute the lack of contractures to the early gestational age because most fetal muscle growth occurs in the last half of gestation.

The selectivity of the involvement of spinal and hypoglossal motor neurons without other changes in neurons of the brain or

spinal cord to suggest hypoxic/ischaemic or metabolic encephalopathy indicates that this motor neuron degeneration is not merely one component of systemic or generalised CNS disease.

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