Severe infantile neuropathy with diaphragmatic weakness and its relationship to SMARD1

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Summary
A group of 13 patients with early onset diaphragmatic palsy in association with a progressive neuropathy is presented. All eight of those tested were found to have mutations in the same gene encoding the immunoglobulin mu-binding protein 2 (IGHMBP2) in patients with spinal muscular atrophy (SMA) with respiratory distress type 1. Six out of these eight patients had either homozygous or compound heterozygous mutations, and two had only a single heterozygous mutation. Detailed analysis of the clinical picture and the neurophysiological and histopathological findings indicated that these patients shared similar characteristics, which were further developed as a set of diagnostic criteria. Some of the most striking of these were early onset of respiratory compromise, a markedly low birth weight, very slow motor nerve conduction velocities and a general decrease in the size of myelinated fibres on sural nerve biopsy. Extensive histological examination of the spinal cord in one patient failed to find any evidence of an SMA. Four out of the five not tested genetically were positive for all diagnostic criteria. None of the cases of early onset neuropathies or spinal muscular atrophies with early respiratory failure reviewed in the literature shares the exact characteristics, but many do have very close similarities. Their classification varies, but the discovery of mutations in IGHMBP2 in cases that are variously classified as SMA plus or severe infantile neuropathy with respiratory distress points to a need for the search for this genetic defect to be widened to include both groups. The fact that we identified other, similar cases of neuropathy and early respiratory failure with and without IGHMBP2 mutations suggests genetic as well as clinical heterogeneity in these infants. It is possible that infants that do not have mutations in the IGHMBP2 gene will be found to have mutations in a similar functioning gene.

keywords: infantile neuropathy; diaphragmatic weakness; respiratory insufficiency; respiratory paralysis; peripheral nervous system diseases

Abbreviations: CMT = Charcot–Marie–Tooth; IGHMBP2 = immunoglobulin mu-binding protein 2; LLN = lower limit of normal; SIANRF = severe infantile axonal neuropathy with respiratory failure; SMA = spinal muscular atrophy; SMARD1 = SMA with respiratory distress type 1; SMN = survival motor neuron

Introduction
Respiratory complications in either hereditary peripheral neuropathy or spinal muscular atrophy (SMA) are usually not seen in the neonatal period. Involvement of the phrenic nerves has been described in adults with Charcot–Marie–Tooth (CMT) type 1 due to the chromosome 17 duplication, PMP22 and MPZ mutations (Hardie et al., 1990; Tyson et al., 1997). The form of autosomal dominant CMT type 2 associated with diaphragmatic palsy and vocal cord paralysis (CMT 2C) is genetically distinct from CMT 2A, 2B and 2D (Nagamatusu et al., 2000), as well as types CMT 2E, 2F and distal hereditary motor neuropathy type VII (Santoro et al., 2002). It has not been described in the neonatal period, although Dyck et al. (1994) did report that severely affected members of two kindreds could be affected in infancy or childhood. The positive family history will make the diagnosis uncomplicated. A case classified as congenital hypomyelinating neuropathy with diaphragmatic and vocal cord paralysis has been reported (Hahn et al., 2001).

It was believed previously that involvement of the diaphragm made the diagnosis of SMA untenable (see table 1
Rudnik-Schoneborn et al., 1996). Later, the 35th European Neuromuscular Centre workshop on SMA (1996) recognized that involvement of the diaphragm can occur in SMA, although the first report was made in 1977 (Kyllerman, 1977). SMA with diaphragmatic involvement is now classified under the SMA plus types and has been shown not to be associated with the deletion of the survival motor neuron (SMN) gene on chromosome 5 (Zerres and Davies, 1999).

Grohmann et al. (2001) studied 11 patients with a clinical diagnosis of SMA with respiratory distress type 1 (SMARD1) and 21 of their relatives from a total of six families. They found mutations in the gene encoding immunoglobulin mu-binding protein 2 (IGHMBP2). An animal model with early onset neuromuscular degeneration, the neuromuscular disorder mouse, also has mutations in the IGHMBP2 gene, and Grohmann et al. (2001) suggest that this mutation is the cause of SMARD1 in humans.

Our group has been interested in a series of neonates who presented with severe respiratory distress in the first few months of life. They had diaphragmatic palsy and evidence of an axonal neuropathy. Sural nerve biopsies showed a general decrease in the size of myelinated fibres with minimal evidence of axonal degeneration and no indication of demyelination. The prognosis was very poor. Our initial cohort of six patients, presented previously in abstract form (Pitt et al., 1998), has now increased to 13 cases, mostly unrelated. We have been unable to find convincing evidence to classify them as SMA and were interested to determine whether they share the genetic abnormalities of the patients studied by Grohmann et al. (2001). The current paper presents these results, along with detailed analysis of clinical, electrophysiological and histopathological findings.

### Case report

**Case 1**

This boy, the first child of healthy unrelated parents, was born at term by emergency Caesarean section, and weighed 2.06 kg. He presented at 8 days of age, with a history of poor feeding requiring nasogastric tube feeding. Four days later he developed signs of respiratory distress. A chest X-ray showed

<table>
<thead>
<tr>
<th>Case</th>
<th>Age tested (weeks)</th>
<th>Duration of illness (days)</th>
<th>Median nerve action potential amplitude (μV) velocity (m/s)</th>
<th>Ulnar motor velocity (ms)</th>
<th>Medial popliteal amplitude (mV) velocity (m/s)</th>
<th>Phrenic nerve latency (ms)</th>
<th>EMG tibialis anterior</th>
<th>EMG diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>34</td>
<td>31.2</td>
<td>21*</td>
<td>0.5*</td>
<td>11.0*</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>16</td>
<td>11.2(22^*)</td>
<td>24</td>
<td>0.2*</td>
<td>Absent</td>
<td>Discrete IP</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>30</td>
<td>12.5(25^*)</td>
<td>17**</td>
<td>0.45*</td>
<td>Not done</td>
<td>Severely reduced IP</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>24</td>
<td>25*</td>
<td>Not done</td>
<td>24*</td>
<td>0.8*</td>
<td>Discrete IP</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>150</td>
<td>2.0*</td>
<td>Absent</td>
<td>0.2*</td>
<td>Absent</td>
<td>Discrete IP</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>11</td>
<td>Absent</td>
<td>25*</td>
<td>Not done</td>
<td>Fibrillation potentials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>190</td>
<td>9.1(16^*)</td>
<td>Absent</td>
<td>0.2*</td>
<td>Absent</td>
<td>Discrete IP</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>28</td>
<td>11(26^*)</td>
<td>Absent</td>
<td>0.16*</td>
<td>No response (technical?)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>180</td>
<td>Absent</td>
<td>Absent</td>
<td>0.04*</td>
<td>Severe reduced IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>70</td>
<td>Absent</td>
<td>22*</td>
<td>0.04*</td>
<td>5.8</td>
<td>Discrete IP + neurogenic units</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>42</td>
<td>Absent</td>
<td>19**</td>
<td>Absent</td>
<td>Absent</td>
<td>Discrete IP + neurogenic units</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>70</td>
<td>Absent</td>
<td>Not tested</td>
<td>2.1</td>
<td>No response (technical?)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>16</td>
<td>21</td>
<td>Absent</td>
<td>33</td>
<td>1.57*</td>
<td>Absent</td>
<td>Fibrillation potentials</td>
<td></td>
</tr>
</tbody>
</table>

For sensory and motor conduction velocities, * = mildly abnormal < lower limit of normal range (LLN) but > 70% LLN; ** = severely abnormal <70% of LLN. For amplitudes of motor and sensory responses, * = abnormal; fibrils = fibrillation potentials; IP = interference pattern. For phrenic nerve latency, * = above upper limit of normal.
bilateral diffuse shadowing. By 11 weeks of age he remained persistently tachypnoeic, was failing to thrive and was referred for further investigation.

On admission, his weight was below the 10th centile, he had grunting respiration and had only a weak cry. He was tachypnoeic, with paradoxical movement of his chest and abdomen, and required oxygen by headbox to maintain normal arterial oxygen saturation. He had anti-gravity movement of his limbs, but his deep tendon reflexes were depressed. Twenty-four hours after admission, his condition deteriorated and required ventilatory support. Chest radiograph showed a raised central portion of his diaphragm and, on screening, there was paradoxical movement of the left hemidiaphragm with little movement of the right, medial leaflet. Results of neurophysiological investigations and nerve biopsies are shown in Tables 1 and 2.

Other investigations, including sweat test, white cell enzymes, creatine kinase, SMN gene deletion, ECG and CT head scan, were either normal or negative.

He required continuous positive airways pressure support and underwent plication of the diaphragm. At surgery, the diaphragm was noted to be thin and membranous. Despite having had his diaphragm plicated, he remained ventilator dependent. Over the next month, he developed progressive distal weakness and lost his ankle tendon reflexes.

At 4 months of age, his distal limb muscles were weak and wasted, without anti-gravity power; proximal limb muscles were affected to a less severe extent. After discussion with his parents, ventilatory support was withdrawn and he died. Permission for post-mortem was declined.

### Patients and methods

#### Patients

All 13 patients (9 males and 4 females) presented to the intensive care units of Great Ormond Street Hospital for Children between 1989 and 2001 with early onset respiratory distress. The majority (11 out of 13) were seen between 1996 and 2001. Two were sisters, one seen in 1989 (Case 4) and the second in 2001 (Case 12). Case 7 had a 6-year-old sister who was being ventilated in Bahrain with a presumed diagnosis of SMA type 1 with a deletion in the SMN gene. Case 13 had a brother with a histopathological diagnosis of CMT type 1 but without genetic confirmation.

Ethics approval was obtained from the joint medical and ethics committee at Great Ormond Street Hospital and The National Hospital for Neurology and Neurosurgery to carry out this clinical and genetic study.

### Neurophysiological methods

Nerve conduction studies and EMGs were performed on a Dantec Counterpoint EMG machine or a Medelec MS6 using techniques as described by Payan (1997). Needle EMG was performed using a fine concentric needle electrode (needle diameter 0.3 mm, recording area 0.019 mm²). Phrenic nerve stimulation was performed using the technique of Markand et al. (1984). The technique of Bolton et al. (1992) was used for needle EMG of the diaphragm.

Normal values for nerve conduction velocities were taken from Raimbault (1988). The lower limit of the amplitude of the median nerve action potential, from wrist to elbow, was 10 \( \mu \)V, and the medial popliteal compound muscle action potential from abductor hallucis was 2.0 mV. These were calculated from the values in ‘presumed normal’ subjects who had been examined in the department over the last 10 years. The phrenic nerve latencies were obtained in the same way and showed a marked decline with age, the upper limit at 3 months being ~7.8 ms, falling to ~5 ms at 20 months.

Chronic denervation with reinnervation was diagnosed when a reduced interference pattern was seen with large amplitude, high-firing polyphasic units and long duration.
units. Acute denervation required the presence of fibrillations in a significant number of sites in the muscle. The nerve conduction velocities were recorded as severely abnormal if they were below 70% of the lower limit of normal (LLN) (modified by the Ad Hoc Committee of the American Association of Neurology recommended criteria for demyelination; Ad Hoc Subcommittee, 1991) and mildly abnormal if above this level but still below the LLN. The skin temperature was above 30°C in all cases.

**Histopathological methods**

Sural nerve biopsies, as well as sural and mixed nerves (popliteal or peroneal) taken post-mortem, were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer. After overnight fixation, 1-mm-thick slices were cut for processing and embedding in resin for semithin and ultrathin sections; the remaining length was processed into unpolymerized resin for later separation of nerve fibres (teasing) in complete resin. Quantitative methods on sural nerve biopsies were as follows:

**Myelinated fibre density from 1 μm sections**

These methods are similar to those described in a previous paper (Jacobs and Love, 1985). Briefly, at least 2000 myelinated fibres from several fascicles were counted at the microscope at a magnification of ×250 using a squared eyepiece graticule. Fascicular areas were measured using a microscope with an extension tube positioned over the bitpad of a Minimop image analyser. A cursor with a light-emitting diode, visible in the microscope, was used to trace round the inner edge of the perineurium of each fascicle. The density of myelinated fibres was calculated.

**Unmyelinated axon density**

Unmyelinated axons were counted in electron micrographs enlarged to about ×10 000 (calibrated with a grating replica) and covering an area of ~0.01 mm² in each nerve. Unmyelinated axons were distinguished from Schwann cell processes by their circular or near-circular outline, their paler cytoplasm often with numerous microtubules and filaments and by the absence of ribosomes.

**Myelinated fibre diameter**

Representative fields from at least two fascicles were photographed and enlarged to just under ×3000, the exact magnification being obtained by calibration. Using the Minimop, areas enclosed by the inner and outer perimeters of the myelin sheath were traced and the data stored in an interfaced computer. Fibre and axon diameters were derived from area measurements assuming circularity, and g-ratios

**Table 3 Clinical and radiological findings**

<table>
<thead>
<tr>
<th>Case</th>
<th>Birth weight (kg)</th>
<th>Gestation (week)</th>
<th>Presenting symptoms (age)</th>
<th>Onset to ventilation</th>
<th>Neurological signs at presentation</th>
<th>Diaph X-ray</th>
<th>Age at death (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.06</td>
<td>37</td>
<td>Poor feeding (8 days), failure to thrive</td>
<td>28 days</td>
<td>Reduced reflexes</td>
<td>R &amp; L</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>2.9</td>
<td>40</td>
<td>Respiratory arrest (1.25 months)</td>
<td>4 days</td>
<td>No wasting, hypotonia or fasculation</td>
<td>R</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>40</td>
<td>Respiratory failure (28 days)</td>
<td>1 days</td>
<td>Not recorded</td>
<td>R &amp; L</td>
<td>Died abroad</td>
</tr>
<tr>
<td>4</td>
<td>2.33</td>
<td>40</td>
<td>Feeding difficulty (2.5 months)</td>
<td>7 days</td>
<td>Hypotonia only</td>
<td>R &amp; L</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>1.89</td>
<td>40</td>
<td>Respiratory tract infection (2.5 months)</td>
<td>7 days</td>
<td>None</td>
<td>R &amp; L</td>
<td>Died abroad</td>
</tr>
<tr>
<td>6</td>
<td>2.83</td>
<td>42</td>
<td>Tachypnoea and feeding difficulty (1 week)</td>
<td>7 weeks</td>
<td>None</td>
<td>R</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>*</td>
<td>40</td>
<td>Hypotonia (3–4 months)</td>
<td>Never ventilated 1 weeks</td>
<td>Hypotonia with proximal wasting</td>
<td>Normal</td>
<td>Died abroad</td>
</tr>
<tr>
<td>8</td>
<td>1.98</td>
<td>40</td>
<td>Feeding difficulty (2.75 months)</td>
<td>2 months</td>
<td>Distal weakness with wasting</td>
<td>R</td>
<td>6.5</td>
</tr>
<tr>
<td>9</td>
<td>1.16</td>
<td>35</td>
<td>Feeding difficulty (3 months)</td>
<td>Immediate</td>
<td>Hypotonia only</td>
<td>R &amp; L</td>
<td>9.5</td>
</tr>
<tr>
<td>10</td>
<td>2.3</td>
<td>38</td>
<td>Respiratory distress (1 month)</td>
<td>Immediate</td>
<td>Hypotonia only</td>
<td>R</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>*</td>
<td>40</td>
<td>Respiratory failure (2 months)</td>
<td>Immediate</td>
<td>None</td>
<td>R &amp; L</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
<td>35</td>
<td>Apnoeas (2 months)</td>
<td>Immediate</td>
<td>Hypotonia</td>
<td>R</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>3.2</td>
<td>40</td>
<td>Failure to thrive, stridor, hypotonia and feeding difficulty (4 months)</td>
<td>Immediate</td>
<td>Talipes and hypotonia</td>
<td>R &amp; L</td>
<td>Alive, ventilator dependent</td>
</tr>
</tbody>
</table>
(axon: fibre diameter) were calculated. At least 400 fibres were measured in each nerve.

Unmyelinated axon diameter

The perimeter of unmyelinated axons was traced on micrographs at about ×7000, accurately calibrated, using the Minimop; diameters were calculated from axonal area assuming axonal circularity. At least 400 axons were measured in each nerve.

Brain, cord and muscle biopsies

Where autopsy was carried out, brains and spinal cords were immersion fixed in formalin for a minimum of 1 week, then blocked for paraffin processing and histological examination utilizing conventional staining with haematoxylin–eosin, luxol fast blue–cresyl violet and immunohistochemistry for glial fibrillary acidic protein (GFAP).

Muscle biopsies were oriented and snap frozen for cryostat sectioning at 8 μm. The sections were stained with haematoxylin–eosin, the modified Gomori trichrome stain, for fat, glycogen and mitochondrial enzyme activity and to demonstrate fibre types with an ATPase method with and without acid preincubation.

Genetics methods

Genetic sequencing

DNA was extracted by standard phenol/chloroform extraction method, a routine automated technique carried out in the genetics laboratory from blood or muscle biopsy samples. The deletion in the SMN gene was excluded in all families, as type I SMA was a differential in all cases.

The 15 exons and flanking intronic regions of the IGHMBP2 gene were amplified by the PCR using oligonucleotide primers purchased commercially (Invitrogen). Primers have been described previously. PCR was carried out in a total volume of 50 μl, which contained 20 ng of DNA, 0.2 mM dNTPs, 1 unit of TaqGold polymerase, 1.5 mM MgCl₂, 75 mM Tris–HCl pH 9.0, 20 mM (NH₄)₂SO₄, 0.01% Tween 20 and 50 pmol of each primer. PCR was performed on a Perkin-Elmer 9700 thermal Cycler (Perkin Elmer, Applied Biosystems, Foster City, CA). The cycling consisted of denaturation at 94°C for 15 min, followed by 25 cycles of 94°C for 30 s, 60–50°C touching down protocol for 30 s and 72°C for 30 s. After that, 12 cycles of constant annealing temperature at 50°C and a final amplification at 72°C for 10 min was carried out. PCR fragments were checked on a 1% agarose gel. The PCR products were purified by using a Qiaquick purification kit (Qiagen, Hilden, Germany) and resuspended in 50 μl of deionized water. For each exon, 100 ng of amplified product was sequenced using forward and reverse primers and a BigDye Terminator cycle sequencing kit (Perkin-Elmer). Sequencing was performed on an ABI377 automated sequencer. Sequence alignment and analysis was carried out with Sequence Navigator (Perkin-Elmer).

Mutation and polymorphism analysis

In addition to the pathogenic mutations identified in the IGHMBP2 gene, a number of single nucleotides polymorphisms (SNPs) were identified throughout the gene. The mutations were characterized in families and controls by re-sequencing affected individuals and sequencing 50 unaffected control patients (Table 3). This allowed clear differentiation between the presence or absence of the mutation or SNPs. A number of nucleotide repeats were identified within the introns of the IGHMBP2 gene. None of the pathogenic mutations identified in Table 3 was found in controls.

Results

Patient details

Details of patients are shown in Table 3. No case was symptomatic at birth, except Case 3, who had grunting
respirations. Decisions to withdraw treatment or transfer prevented detailed follow-up in most cases. Where serial neurological observations were made (Cases 1, 2, 6 and 8), weakness and subsequent wasting developed over a period of weeks and showed a distal to proximal progression.

**Neurophysiology**

Results of neurophysiological investigations are shown in Table 1. The most severe abnormalities were markedly reduced motor conduction velocities, particularly in the legs, and a very marked reduction or loss of the compound muscle action potential. Studies of the sensory fibres showed similar abnormalities of conduction velocity and compound action potentials, but of a much milder type. The EMG in each case revealed a denervation pattern only in distal muscles initially. An EMG of the diaphragm was made in four cases (9–12) and confirmed the presence of denervation in three. This was confirmed by post-mortem biopsy in Case 10.

**Histopathology**

**Nerve biopsy results**

**Qualitative findings**

Sural nerve biopsies from Cases 1–11 showed a similar appearance, compared with age-matched control sural nerves. The myelinated fibres were small (Fig. 1). There was no evidence in 1 μm resin sections of fibre degeneration, regeneration or demyelination. In a few cases, a very occasional degenerating fibres was seen. There was no evidence of demyelination or remyelination in any of the teased nerves. Cases 12 and 13 showed different features: in Case 12, there was significant evidence of degeneration reflected in the reduced myelinated fibre density (Table 2); and in Case 13, the myelinated fibre size spectrum was normal, with only very occasional evidence of fibre degeneration.

Electron microscopy of Cases 1–11 and 13 revealed a few denervated Schwann cell processes (Bands of Bungner), evidence of occasional myelinated fibre degeneration. In Case 12, there was frequent evidence of fibre degeneration.

There was no evidence of loss of unmyelinated axons. The ultrastructure of axons of myelinated and unmyelinated axons appeared normal: in particular, neurofilaments seemed to be present at normal densities. In mixed nerves taken post-mortem (Cases 2, 4 and 6), the motor components were identified from the larger size of the motor fibres. In resin sections, there was some evidence of fibre degeneration in these motor fibres; Fig. 2 shows a reduced density of myelinated motor fibres. Electron microscopy confirmed the presence of numerous Bands of Bungner in such regions, indicating earlier myelinated fibre degeneration.

**Quantitative findings in sural nerve biopsies**

Control data were obtained from sural nerves of four infants aged 0 and 3 days, and 3 and 9 weeks, removed within 24 h of death from cases without a history of peripheral nerve disease (Jacobs and Love, 1985). Inclusion of controls rather younger than our patients emphasizing the marked difference between the two groups. Table 2 summarizes the quantitative findings.

Fascicular areas: fascicular areas were generally smaller than those of the controls, and this is probably explained by the smaller size of the fibres.

Myelinated fibre density: there were no obvious differences between myelinated fibre densities in control sural nerves and nerve biopsies from Cases 1–11. The reduced density in Case 12 is associated with more marked evidence of degeneration of myelinated fibres.

Myelinated fibre size: it is justifiable to use the mean fibre diameter measurement for purposes of comparison when
there is no evidence of fibre loss as in Cases 1–11. Table 2 shows significantly reduced mean fibre diameters in all except Case 13. There is a similar significant reduction in size of the axons of these myelinated fibres, again excepting Case 13. In histograms, both the myelinated fibre diameter size spectrum (Fig. 3) and their axon diameters show a marked shift to the left compared with the control nerves.

Myelin sheath thickness: the slope of the regression line relating myelin sheath thickness and fibre diameter was compared between controls and Cases 1–6. Mean values of 0.108 ± 0.022, (n = 5, controls) and 0.103 ± 0.022 (n = 6, patients) were not different statistically (Levenes test for equality of variances, $F = 0.009, P = 0.926$), confirming that the myelin sheaths were of appropriate thickness for the size of axon.

Unmyelinated axon density: these are within normal limits in Cases 1, 3, 4 and 5. Mean unmyelinated axon diameters from controls and these cases were similar.

**Brain, cord and muscle biopsy results**

Examination of serial sections of the spinal cords of Cases 4 and 6 showed no abnormality, in particular no loss or changes in anterior horn cells or accumulation of microglia (Fig. 4). Dorsal root ganglia from cervical, thoracic and lumbar regions showed no abnormalities. Diaphragmatic muscle showed denervation in Case 10 but no abnormality in Case 6, although the intercostal muscles were denervated.

Ten of the patients had muscle biopsies. Case 6 had two, the first in vastus lateralis, which showed no diagnostic features, the second in medial gastrocnemius, which showed chronic denervation. The site of the biopsy was medial gastrocnemius in four cases, all of which showed changes reported as consistent with chronic denervation, and vastus lateralis in three, all of which showed non-specific changes. The exact site was not recorded in four examinations and the conclusions made were as follows: possibly neurogenic, denervation of unusual type, chronic denervation and acute denervation.

**Genetic analysis**

Genetic analysis was carried out on eight cases, and the results are shown in Table 4. As can be seen, five out of the eight cases have compound heterozygous mutations, Case 7 has a homozygous deletion and Cases 2 and 6 has a single heterozygous deletion. Cases 2 and 6 may have an unidentified deletion involving one or more exons or a cryptic splice site change, or they may have a mutation in another gene acting in parallel to the *IGHMBP2* gene. The pathogenicity of single heterozygous changes is not proven in this context, although the change was not present in 50 British controls. Case 7 is from Bahrain; ethnically matched controls were not available, but this change was not present in 50 British, five Pakistani, four Indian and one Iranian control. The mutations in exons 2, 7, 8 and 10 were not identified in 50 British controls; detailed ethnicity was not available in these cases, but they were clinically assessed in the UK. The position of the mutations on the *IGHMBP2* gene are shown in Fig. 5, which also shows the mutations identified by Grohmann *et al.* (2001).

**Proposed diagnostic criteria**

The results of our investigations suggest that the cases shared common clinical, neurophysiological and histopathological
characteristics, which might perhaps allow for their more accurate diagnosis and also distinguish them from other reported cases. We judged the cases against clinical, histological and neurophysiological criteria. The criteria chosen are shown below.

Clinical criteria
The clinical criteria were (i) low birth weight below the 3rd centile; (ii) onset of symptoms within the first 3 months; (iii) diaphragmatic weakness either unilaterally or bilaterally; (iv) ventilator dependence within less than one month of onset with an inability to wean; and (v) absence of other dysmorphology or other conditions.

Histopathological criteria
The histopathological criteria were (i) a shift to the left of myelinated fibre size in sural nerve biopsies (since myelin sheath thickness is found to be appropriate for axon size, the fibre size change would have originated from the axon); (ii) minimal evidence of ongoing myelinated fibre degeneration in biopsies taken up to 3–4 months; and (iii) no evidence of regeneration or of demyelination which might account for the change in fibre size.

EMG criteria
EMG criteria were fulfilled if, in any of the investigations performed, there was (i) evidence of distal denervation acute or chronic (ii) evidence of severe slowing (<70% of LLN) in one or more nerves (motor or sensory).

The Venn diagram (Fig. 6) shows the criteria that the cases met, with the reasons for the cases not fulfilling a particular criterion and also whether the cases were tested for the mutations.
All of the eight cases tested showed mutations in the gene encoding IGHMBP2 located on chromosome 11q13.2-q13.4. In two cases, only a single heterozygous mutation was found; and even though these mutations were not found in controls, the significance of these single changes are uncertain, as the pathogenicity of these mutations is not proven in the absence of a mutation on the other allele. Four out of the remaining five cases who were not tested fulfilled all the clinical, physiological and pathological criteria for classification, and the other, Case 4, was the elder sibling of Case 12, who was positive. Although not possible to prove, it would be anticipated that they might also have tested positive. The location of the mutation positions was rarely the same in our group compared with those of Grohmann et al. (2001). All the identified mutations were likely to be pathogenic, except perhaps for the two cases with single heterozygous mutations as discussed above: they either changed a conserved amino acid, caused a mutation that produces a stop codon or caused a deletion that resulted in a truncated protein. None of the mutations was identified in controls.

Infants and neonates who present with severe respiratory distress from diaphragmatic palsy have been described. They are defined as either diaphragmatic SMA (Zerres and Davies, 1999) or severe infantile axonal neuropathy with respiratory failure (SIANRF) (Wilmshurst et al., 2001). Some have slow conduction velocities and many do very badly, either dying early or remaining ventilator dependent. We initially felt that our patients had a distal SMA, but the spinal cord was entirely normal histologically in Case 6 and consistent sural nerve changes were seen in all cases. It seemed more appropriate they were reclassified as a severe infantile polyneuropathy. Grohmann et al. (2001) regard autosomal recessive SMARD1 as synonymous with SIANRF (Wilmshurst et al., 2001). The criteria presented here may help clinicians to identify similar cases.

There are 39 cases in the literature [Mellins et al. (1974), two cases; McWilliam et al. (1985), four cases; Schapira and Swash (1985), two cases; Bertini et al. (1989), five cases; Appleton et al. (1994), one case; Novelli et al. (1995), two cases; Grohmann et al. (1999), nine cases; Geller et al. (2000), three cases; Mercuri et al. (2000), one case; Grohmann et al. (2001), three new cases in addition to those reported elsewhere; Mohan et al. (2001), two cases; and Wilmshurst et al. (2001), five cases]. Sex was given in only 26 cases (14 males and 12 females). Thirteen out of the 29 with family history were positive. Only six out of the 20 with complete clinical details fulfilled all criteria, but the birth-weight was low in 12. Seven out of the 17 with neurophysiological details fulfilled our criteria. Often the slowing in nerve conduction velocity was not as severe as we had seen. The cases that were closest to ours were Mellins et al. (1974), case 1; Bertini et al. (1989), case 3; Appleton et al. (1994), single case; Geller et al. (2000), case 3; and Wilmshurst et al. (2001), cases 1 and 5. All had low birth weight and six out of the seven remaining criteria.

Insufficient data prevented the same analysis of the histological findings. The sural nerve biopsy from Appleton’s case (Appleton et al., 1994) was reviewed in our laboratory and found to have the same changes as the majority of our group. Most importantly, the sural nerve biopsy findings were not specific. Identical changes were found in five other patients, four of whom had tested negative.

**Discussion**

All of the eight cases tested showed mutations in the gene encoding IGHMBP2 located on chromosome 11q13.2-q13.4. In two cases, only a single heterozygous mutation was found; and even though these mutations were not found in controls, the significance of these single changes are uncertain, as the pathogenicity of these mutations is not proven in the absence of a mutation on the other allele. Four out of the remaining five cases who were not tested fulfilled all the clinical, physiological and pathological criteria for classification, and the other, Case 4, was the elder sibling of Case 12, who was positive. Although not possible to prove, it would be anticipated that they might also have tested positive. The location of the mutation positions was rarely the same in our group compared with those of Grohmann et al. (2001). All the identified mutations were likely to be pathogenic, except perhaps for the two cases with single heterozygous mutations as discussed above: they either changed a conserved amino acid, caused a mutation that produces a stop codon or caused a deletion that resulted in a truncated protein. None of the mutations was identified in controls.

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**Fig. 6** Venn diagram of the cases identified by their case numbers, categorized by their assessment against the three sets of criteria. The cases within shaded boxes are those tested genetically (Cases 1, 2, 6–9, 12 and 13).
for mutations in the \textit{IGHMBP2} gene (one was not tested). These four patients presented at birth or soon afterwards and needed immediate ventilatory support. Only one had a low birth weight, but also had complicated heart disease and was dysmorphic, and was diagnosed as having had an axonal sensorimotor neuropathy. Of the others, one case had grade II intraventricular haemorrhage, deficiency of the anterior ribs but no diaphragmatic abnormality and the EMG was suggestive of a neuromuscular junction abnormality. Another baby was clearly dysmorphic and had microgastria and was thought to have a severe SMA on nerve conduction studies. The final infant had no other somatic abnormalities but had neurophysiological changes of a sensorimotor neuropathy. The diaphragm was not involved in any case and none showed the characteristic severe slowing of motor nerve conduction. Therefore, the condition on histopathological criteria is heterogeneous.

The recent case described by Hahn et al. (2001), the first to report phrenic nerve involvement in a congenital hypomyelinating neuropathy, may be another case, as it has many of the characteristics of our cases, including low birth weight, absence of large diameter myelinated fibres and distal denervation. Interpretation of a neuropathy as hypomyelinating may be difficult in a nerve biopsy taken at the age of 1 month, when myelination is still incomplete. Quantitative comparison with age-matched controls is required to confirm the degree of myelination.

It seems likely that SMARD1/SIANRF are descriptions of a condition with variable clinical expression, of which a proportion will have mutations in the \textit{IGHMBP2} gene. The recent linkage study of Viollet et al. (2002) in a family with a SMARD phenotype that was negative for the \textit{IGHMBP2} gene identified a locus close to SMARD1 on 11q. Its is likely that families that are negative for \textit{IGHMBP2} will have a defect at this locus that is likely to affect a gene functionally similar to \textit{IGHMBP2}.

The differing pathological involvement reported in the peripheral nerves and spinal cords may have a parallel in severe congenital SMA (Zerres and Davies, 1999). On molecular analysis, a large deletion including the \textit{SMN} telomeric and \textit{NAIP} genes is seen in most families with this condition linking them unequivocally to SMA 5q. Yet, on neurophysiological testing, there is a lack of sensory and motor nerve excitability. Moreover, the sural nerve biopsy shows typical signs of axonal degeneration. The histological examination of the spinal cord may not show a marked reduction in the number of motor neurones, despite most severe clinical manifestations.

The absence of the largest diameter axons in the sural nerve biopsy explained the low velocities in sensory nerves. The velocity decrease on nerve conduction studies was so slow that the initial impression was of demyelination as the underlying pathology. When demyelination is encountered in the neonatal period, it is most unusual to obtain sensory responses, which in these cases could often be found. High stimulation intensities are also needed (Bolton, 1996). Although it is well recognized that some slowing may occur in the classical SMA patients, slowing to <70% of the LLN remains an exclusion criterion. The combination of the slow motor nerve conduction velocity and severe distal denervation is unusual and was a signature of the condition. It was not possible to make the same correlation between the motor nerve conduction studies and the histopathology of motor nerves.

The impression was not one of loss of nerve fibres with the accompanying changes of axonal degeneration, but rather that the nerve fibres had not developed. Myelinated axons did not attain their expected size for age. Axon calibre is associated with neurofilament density, which in the sural nerves appeared normal; the total number of neurofilaments produced is therefore less than normal. \textit{IGHMBP2} colocalizes with RNA-processing machinery in cytoplasm and nucleus, but no information about its specific effects is available. However, the fact that there were similar sural nerve findings in other cases that were clinically and genetically distinct may suggest that the \textit{IGHMBP2} gene is not involved in the axon changes. It is possible that the sural nerve changes simply reflect an early onset neuromuscular disorder and are not specific for any particular cause. The changes seen in the child with abnormalities suggesting a neuromuscular junction abnormality are otherwise impossible to reconcile.

With the genetic studies presented, it is unlikely our cases are examples of the infantile neuropathy initially described by Ouvrier et al. (1981) and to which more cases have been added by Gabreels-Festen et al. (1991). Our cases are also different in their clinical, genetic and morphological aspects from autosomal dominant CMT type 2, which also show a distinct loss of the largest diameter myelinated fibres with a shift in the histogram to the left. Regenerative features are almost absent. With the identification of \textit{IGHMBP2} mutations, it will be interesting to screen all cases presenting with similar clinical and neurophysiological findings, irrespective of whether they are considered a neuropathy or an SMA. This will help further define the phenotype and also allow examination to see whether there is a phenotype/genotype effect explaining some of the features. Given the clinical and genetic heterogeneity in this patient group, identification of further genes will also help us to define a genetic classification.

References


