Phenotypical features of the p.R120W mutation in the *GDAP1* gene causing autosomal dominant Charcot-Marie-Tooth disease

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Abstract Mutations in the *ganglioside-induced-differentiation-associated-protein 1* gene (*GDAP1*) can cause Charcot-Marie-Tooth (CMT) disease with demyelinating (CMT4A) or axonal forms (CMT2K and ARCMT2K). Most of these mutations present a recessive inheritance, but few autosomal dominant *GDAP1* mutations have also been reported. We performed a *GDAP1* gene screening in a clinically well characterized series of 81 index cases with axonal CMT neuropathy, identifying 17 patients belonging to 4 unrelated families in whom the heterozygous p.R120W was found to be the only disease-causing mutation. The main objective was to fully characterize the neuropathy caused by this mutation. The clinical picture included a mild-moderate phenotype with onset around adolescence, but great variability. Consistently, ankle dorsiflexion and plantar flexion were impaired to a similar degree. Nerve conduction studies revealed an axonal neuropathy. Muscle magnetic resonance imaging studies demonstrated selective involvement of intrinsic foot muscles in all patients and a uniform pattern of fatty infiltration in the calf, with distal and superficial posterior predominance. Pathological abnormalities included depletion of myelinated fibers, regenerative clusters and features of axonal degeneration with mitochondrial aggregates. Our findings highlight the relevance of dominantly transmitted p.R120W *GDAP1* gene mutations which can cause an axonal CMT with a wide clinical profile.

Key words: Charcot-Marie-Tooth disease; CMT2K; ADCMT; *GDAP1* mutations.

Introduction

Charcot- Marie-Tooth disease (CMT) is a genetically heterogeneous group of inherited motor and sensory neuropathies. Molecular studies have shown extensive genetic heterogeneity in CMT neuropathies with an ever-growing list of causative mutations and loci (*Pareyson and Marchesi, 2009*). Mutations in the *ganglioside-induced-differentiation-associated protein 1* (*GDAP1*; MIM 606598) gene 8q21 have been reported in CMT patients with demyelinating (CMT4A; MIM 214400) (*Baxter, et al., 2002*) and axonal forms (CMT2K and ARCMT2K; MIM 607831) of the disease (*Cuesta, et al., 2002*). Inheritance in most CMT causative *GDAP1* mutations is autosomal recessive (*Boerkoel, et al., 2003; Sevilla, et al., 2003*) characterized by a severe phenotype with early disease onset and rapid progression to important disability in the second or third decade. In recent reports certain mutations have been shown to segregate as an autosomal dominant (AD) trait with a later disease onset and a mild phenotype (*Claramunt, et al., 2005; Chung, et al., 2008a; Cassereau, et al., 2009*) being the p.R120W missense mutation the most prevalent one (*Claramunt, et al., 2005; Cavallaro, et al., 2009*).

In a previous study we reported a series of autosomal recessive or sporadic *GDAP1* related neuropathies (*Sevilla, et al., 2008*). The extension of the screening for mutations in the *GDAP1* gene to autosomal dominant families with axonal CMT has allowed us to recognize 17 patients belonging to 4 unrelated families in whom the only detected mutation was *GDAP1* p.R120W in heterozygosis, two of them were only known by history and genetics. The main objective of the present study was to characterize clinically, electrophysiologically and pathologically this form of CMT neuropathy, designated CMT2K. Additionally a systematic MRI investigation was performed in these patients so as to identify specific patterns of muscle involvement,

as has been described in other types of CMT disease (*Chung, et al., 2008b; Gallardo, et al., 2006*).

Material and Methods

Study subjects

We investigated a clinically well characterized series of 81 patients who presented with axonal CMT. A mutational screening of the more frequent axonal CMT genes, *MFN2*, *GJB1, GDAP1* and *MPZ*, and some of the rarer ones, *NEFL*, *HSP22* and *HSP27*, has been carried out. Causative mutations have been recognized in 34 probands and in 4 of them, the *GDAP1* p.R120W mutation was the only one present. We have identified 17 patients belonging to these four families with the *GDAP1* p.R120W (c.358C>T) mutation in which disease was inherited as an autosomal dominant trait. The pedigrees are displayed in Fig. 1, family B was previously reported (Claramunt, et al., 2005). The families were unrelated, and do not *proceed from the same Spanish region*. All protocols performed in this study complied with the ethics guidelines of the institutions involved. All patients and relatives were aware of the investigative nature of the studies and gave their consent.

Genetic analyses

Blood samples were drawn from the patients and relatives after informed consent and in accordance with the Helsinki declaration. Genomic DNA was obtained by standard methods from peripheral white blood cells. Mutation analysis of the *GDAP1* gene was performed by amplification of the 6 exons and their intronic

flanking sequences using primers previously described. The PCR products were analyzed by DHPLC (*Denaturing High Liquid Chromatography*, Transgenomic WAVE® System) and the anomalous patterns were investigated by automated sequencing (ABI Prism 3130xl, Applied Biosystems). When possible, segregation analyses were performed.

Linkage analysis was carried out under the assumption of autosomal dominant inheritance, full penetrance, and equal frequency of marker alleles. Pairwise LOD scores were calculated using the MLINK program version 5.1 of the FASTLINK package 2.1 (*Lathrop and Laouel, 1984*). Haplotype analyses at the GDAP1 locus was carried out on the basis of cen_*D8S279-D8S286-D8S551*-c.507T>G-*D8S1474-D8S1829-D8S84*_tel as previously described (*Claramunt, et al., 2005*).

Clinical and electrophysiological assessments

All probands and individuals at-risk were examined except patients B-II2 and B-III2. The clinical assessment included strength, muscle atrophy, sensory loss, reflexes, foot deformities as well as a general and neurologic examination. Muscle strength was graded using the standard Medical Research Council (MRC) scale. CMT neuropathy score (CMTNS) was applied to determine neurological impairment: mild (CMTNS≤10 points), moderate (CMTNS 11–20) and severe (CMTNS 21–36) (*Shy, et al., 2005*). The functional disability scale (FDS) was used to measure the disability status (*Birouk, et al., 1997*). Data from family B was updated except for patients B-II2 and B-III2, who were not available for this study.

Electrophysiological studies were performed in 14 of the patients following the same protocol that was described previously (*Sevilla, et al., 2003*).

Magnetic resonance imaging

MRI was performed on the feet and distal legs of 8 patients in a supine position using a 1.5 T MR platform (Siemens Avanto, Erlangen, Germany). The following protocol was used in all patients: axial and coronal T1-weighted TSE (turbo spin echo), STIR, and T1 fat-saturation images before and after Gadolinum-DTPA (Magnevist, Schering, Germany) administration were obtained of both legs and feet. Only one imaging plane was acquired in each region after contrast administration.

The four classic anatomical compartments were used to evaluate calf muscles: anterior compartment (tibialis anterior, extensor hallucis longus, and extensor digitorum longus), lateral compartment (peronei longus and brevis), superficial posterior compartment (soleus and gastrocnemius), and deep posterior compartment (tibialis posterior, flexor digitorum longus and flexor hallucis longus). In axial MR images of lower limbs, fatty infiltration was graded from 0 to 4 as follows: 0, no fat signal in muscle; 1, some fatty streaks; 2, fat occupying a minor part of muscle; 3, similar amount of fat and muscle tissue; 4, fat occupying the greater part of muscle (*Chung, et al., 2008b*).

Nerve biopsies

Sural nerve biopsy was performed in two cases (patients A-II1 and C-II2) when they were 47 and 31 years old respectively, and compared to a 27 year-old multiorgan donor without neuropathic or systemic disease history. Semi-thin sections stained with toluidine blue were prepared for evaluation under a light microscope following the same protocol as described previously (*Sevilla, et al., 2003*). Morphometry of myelinated fibres was performed on high-resolution micrographic

images obtained with a Polaroid DMC digital camera and analyzed by means of Scion image analyst software (http://www.scioncorp.com). Ultra-thin cut samples were contrasted with uranyl acetate and lead citrate for ultrastructural study

Results

Genetic analyses

17 patients from 4 unrelated families carrying the *GDAP1* p.R120W mutation in a heterozygous state were identified. No other pathogenic mutations were detected in other exons or in their flanking intron regions. Autosomal dominant inheritance was confirmed by co-segregation of the *GDAP1* p.R120W mutation with disease in families A, B and C (Fig. 1). In family D only sample from the proposita was available and therefore, a segregation analysis was not possible. Next, we performed a linkage analysis for the *GDAP1* p.R120W mutation in the three multiplex families. The obtained cumulative maximum LOD score (Z_{max}) was of 2.83 (θ=0.00). This Z_{max} ~3 reinforces that the *GDAP1* p.R120W presents an autosomal dominant inheritance. We have constructed haplotypes by analysis of six flanking microsatellite markers, which span ~2.83-Mb around the *GDAP1* locus, and one intragenic SNP. All the chromosomes carrying the *GDAP1* p.R120W mutation share the same haplotype 3-5-G-5-6-6 on the basis of cen_*D8S286-D8S551*-c.507T>G-*D8S1474-D8S1829-D8S84*_tel, suggesting a common origin.

Clinical findings

The clinical characteristics of 15 patients from the four families with the p.R120W missense mutation in the *GDAP1* gene are summarized in table 1. The age in which patients experienced the first symptom ranged between 9 and 65 years

(median 17 years), with disease duration between 5 and 48 years. There was no delay in the acquisition of motor milestones. Four patients (A-II2, A-II3, B-III1 and C-I2) aged 20, 38, 33 and 72 did not complain of any symptom but clinical examination revealed minor signs. Patient A-II2 had mild weakness in toe extension, hyporreflexia and hypoesthesia in lower limbs, patient A-II3 absent ankle reflexes and distal hypoesthesia, patient B-III1 pes cavus, and patient C-I2 absent knee and ankle jerks.

The disease started in the distal lower limbs. Only two patients had mild proximal involvement in the lower limbs (A-I1 and D-II1), being quite disabling in one case. All symptomatic cases presented weakness in ankle plantar flexion (EHL) and toe extension. Weakness in ankle dorsiflexors was present to the same degree as plantar flexors in most symptomatic patients, being the impairment of heel and toe walk quite analogous. Distal upper limb weakness appeared later in the course of the disease and involved the intrinsic hand muscles predominately. At the time of the examination, 12 patients had motor deficits in distal lower limbs, 7 patients in both the distal lower and upper limbs. In one patient (C-II2), the distribution of atrophy and weakness in the lower limbs was asymmetric. ~~Proximal muscle strength was abnormal in lower limbs in 2 patients~~. All patients except two had preserved reflexes in the upper limbs, but hypo/arreflexia in lower limbs and different degrees of lower limb distal atrophy. Foot deformities, including pes cavus and Achilles tendon shortening were also very common. Sensory loss proportional to the motor deficit was present in all symptomatic patients, especially pinprick and vibration in distal lower limbs. Patient A-II2 received chemotherapy for breast cancer ten years ago, but during a 24 year follow-up the disability has followed a progressive course.

The CMTNS scores in the series reflected the wide phenotypic spectrum, ranging from 0-25. There were only two patients that were catalogued in the CMTNS

severe category, both of which had a long disease evolution. Nine patients (60%) were in the mild category and 4 in the moderate. All patients to date have a functional disability scale equal or less than 4 except two patients, patient A-I1 who is mostly wheelchair bound, and patient D-II1 who needs a cane or crutches to walk. Disease progression was slow in the majority of cases but two patients (A-I1 and B-I4) had onset after 40, but developed a relevant functional impairment.

Electrophysiological studies

Table 2 summarizes the nerve conduction and electromyography studies performed. Motor nerve conduction velocities, distal latencies and F-waves were normal in all tested nerves (median MNCV > 54 m/sec in all patients). Ulnar and median CMAP were reduced only in two individuals, while peroneal CMAP was reduced in most affected individuals. Sensory nerve conduction studies showed a reduction of SNAP in all tested patients, but conduction velocities and distal latencies were preserved in nerves with SNAP > 0.5 μv. In the whole series, even in asymptomatic patients, needle electromyography revealed MUAPs (motor unit action potentials) increased in amplitude, duration and polyphasic incidence. Positive sharp waves and fibrillation potentials were not present.

MRI studies

All patients had detectable abnormalities in the MRI consisting in fatty infiltration and/or muscle edema (Table 2).

Intrinsic musculature of both feet showed consistent and bilateral fatty infiltration of the foot muscles in all patients, even in asymptomatic ones (Fig. 2A). The changes were present in all intrinsic foot muscles and were more pronounced in

severely affected patients. All patients had evidence of varying degrees of fatty substitution in the muscles of the calf in concordance with the severity of the phenotype. In any case there was a common pattern: a predominance of fatty substitution distally and in the posterior compartment over the anterolateral one.

Mild cases showed distinct abnormalities in the distal muscles of the calf; high signal intensity on T1-weighted and STIR images, corresponding with fatty substitution and muscle edema respectively. The first muscle affected and the one in which the findings were more prominent was the gastrocnemius, and to a slightly lesser degree the soleus (Fig. 2B), being the rest of the muscles in the calf completely preserved in most mild cases.

In more severe cases the fatty substitution involved all muscle compartments of the calf and muscle edema was no longer present. The posterior compartment was always affected to a greater degree than the anterolateral one with a mean grade of 3.1 vs. 1.5 ($p<0.05$) (Fig. 2C). MRI of the thigh was performed in only 2 patients (A-II1 and D-II1) with a moderate-severe phenotype, existing fatty substitution distally in all the muscles.

Pathologic findings

The two sural nerves biopsies performed revealed similar pathological findings. Semi-thin sections showed a pronounced depletion of myelinated fibres in both nerves (fibre density was 3202/mm^2 in patient A-II1, 5863/mm^2 in patient C-II2, and 9095/mm^2 in the control subject). The histograms representing myelinated fibre size were 'shifted to the left' showing a marked reduction of large-diameter fibres especially in patient A-II1 (fibres >6 μv = 12,3 % in A-II1, 31,5% in C-II2 and 41,7% in control) ~~(Fig. 3)~~. Morphometric data also revealed a considerable proportion of thinly

myelinated fibres with a g-ratio greater than 0.7 (18.2% in A-II1, 20.9% in C-II2 and 5% in control).

Rather frequent regenerative clusters and occasional onion bulb formations were also present (Fig. 3A). In detailed electron-microscope views, onion bulbs were made up of concentric layers of Schwann cell processes adopting a crescent shape and enclosing a central core composed of a regenerative cluster, or less frequently a hypomyelinated or normally myelinated fibre (Fig. 3B). These formations had a limited number of folds (pseudo-bulbs) not reaching the size observed in CMT1A. The regenerative clusters were composed of a group of small myelinated fibres, a large bundle of unmyelinated axons or a combination of the two (Fig. 3E).

On high magnification, myelin compaction always appeared normal. However, axons often presented degenerative features consisting on axolemma retraction with partial or total detachment (Fig. 3C), aggregation of normal and abnormal mitochondria mixed with empty vacuoles, and cytoskeleton dissolution with early disappearance of microtubules (Fig. 3D). Degeneration involved both myelinated and unmyelinated axons with preference for those included in regenerative clusters (Fig. 3F).

Discussion

Autosomal dominant *GDAP1* mutations are exceedingly rare in most published CMT series. Here we present the clinical records belonging to 17 carriers of the *GDAP1* p.R120W mutation from 4 families. They represent 5% of our series of axonal CMT index patients. Whether this indicates a genetic drift in our population or an underrepresentation in other series is a pending question.

It is also noteworthy that all our families shared a common haplotype, which is probably the consequence of a founder effect, like other reported *GDAP1* mutations (*Claramunt et al., 2005*). This mutation has also been detected in two unrelated cases in Belgium and Italy *(Ammar et al., 2003; Cavallaro et al., 2009).* It could be very interesting to construct haplotypes in these two families so as to ascertain if all of them share the same haplotype and therefore, a unique origin could be postulated for the *GDAP1* p.R120W mutation.

The clinical picture comprehends a mild-moderate phenotype with great clinical variability. Disease onset varied, and duration is not clearly related to phenotypic severity. The identification of four practically asymptomatic mutation carriers, one of whom was already age 72, suggests that the p.R120W mutation may have incomplete penetrance. Weakness was first manifested distally in the lower limbs with the peculiarity that ankle dorsi and plantar flexors were impaired to similar degrees, as were tiptoe and heel walking. This picture contrasts with the typical CMT1A (MIM 118220) patients, which usually begin with foot drop due to weakness of ankle dorsiflexors (*Birouk, et al., 1997*), being more similar to patients with late onset *MFN2* (*mitofusin 2*) mutations (CMT2A; MIM 609260) in which there may exist a predominance of ankle plantar flexion weakness and greater difficulty in toe than in heel walking (*Chung, et al., 2008b*).

Comparing the distribution of motor weakness in autosomal dominant patients to patients carrying recessive *GDAP1* mutations is quite a difficult task, due to the severity and the rapid disease progression in the latter. In our series with recessive *GDAP1* mutations (S*evilla, et al., 2008*) characterization of the distal distribution weakness has been possible only in 2 (in one data not published) who had a slightly more indolent course. In these patients the motor weakness in ankle plantar and

dorsiflexion was analogous, as was the impairment of toe and heel walking, findings quite similar to the dominant forms. None of the dominant patients had stridor or voice hoarseness, a common characteristic in recessive forms (*Azzedine, et al., 2003; Senderek, et al., 2003; Moroni, et al., 2009*). Proximal muscles were involved only late in the course of the disease in some patients, causing impairment of independent ambulation.

Nerve conduction studies in our series revealed motor velocities in the axonal range. Needle electromyography exposed giant motor units even in asymptomatic patients. This finding was very consistent in our series and has been already described in another family with the p.C240Y dominantly inherited mutation in *GDAP1* (*Cassereau, et al., 2009*). The physiopathology of this remains unclear, but is consistent with the slowly progressive nature of disease permitting significant collateral sprouting and hence motor unit remodelling.

The pathologic study of the two sural nerve biopsies performed was quite homogeneous and very similar to those described in the recessive *GDAP1* mutations, although the fiber loss was clearly more prominent in the latter (*Sevilla, et al., 2003*). The main abnormalities were loss of myelinated fibres and axonal degenerative features. The presence of regenerative clusters was prominent, and may represent a true reparative process of sprouting after axonal damage, or an inadequate development of myelinated axons. Whatever the origin, these sprouted fibres could account for the high proportion of hypomyelinated fibres reported in the morphometric data. The presence of the small onion bulb formations is not yet explained, but probably do not correspond to a purely demyelinating phenomenon, as the nerve conduction velocities are clearly in the axonal range. These findings have also been reported in other axonal neuropathies like those related to *MFN2*

mutations (*Chung, et al., 2006*).

In our series, the pattern of muscle abnormalities in MRI was quite homogeneous and concordant with disease severity. The main findings described were fatty substitution of affected muscles, atrophy and occasionally edema in subacute muscle denervation (*Fleckenstein, et al., 1993; May, et al., 2000*). These were consistent and present to a greater or lesser degree in all tested patients, even in patients like C-I2 who was asymptomatic, and had no abnormalities in examination except absent lower limb reflexes. The first affected muscles were the intrinsic foot and distal calf muscles, with a clear predominance of the posterior over the anterolateral compartment. Fatty infiltration in the calf sequentially extended from gastrocnemius to soleus muscles and in time to the anterolateral compartment muscles (Table 2, Fig. 2B & 2C). This pattern is quite similar to that reported in late-onset CMT2A (*Chung, et al., 2008b*) but in this type the soleus muscle in the superficial posterior compartment was the earliest and most severely affected muscle. On the other hand the pattern is quite different to that in CMT1A (*Gallardo, et al., 2006*) where there is a predominance of fatty substitution in the anterolateral compartment of the calf. These differences in the pattern of lower leg muscle involvement in diverse types of CMT are one of the more solid reasons to consider MRI as an important tool for phenotypic CMT characterization. In any case further studies are needed to confirm if the preferential involvement of the posterior superficial compartment beginning with the gastrocnemius is specific for *GDAP1* mutations.

Most of *GDAP1* mutations co-segregate with CMT in an autosomal recessive manner, whereas autosomal dominant *GDAP1* mutations are rare. Mutations causative of an axonal CMT neuropathy with both dominant and recessive patterns

of inheritance have been reported in three other genes: *NEFL* (*Abe, et al., 2009; Yum, et al., 2009*), *HSP27* (*Houlden, et al., 2009*) and *MFN2* (*Nicholson, et al., 2008; Calvo, et al., 2009*). To date six *GDAP1* missense mutations with autosomal dominant inheritance pattern have been reported: p.R120W, p.T157P, p.Q218E, p.C240Y, p.P274L and p.H123R (*Claramunt, et al., 2005; Chung, et al., 2008a; Cassereau, et al., 2009; Cavallaro, et al., 2009*). Each of these mutations have been described in only one family except the p.R120W and the p.H123R changes: p.H123R has been identified in CMT patients from two unrelated families (*Cavallaro, et al., 2009*) and p.R120W in six unrelated families including those of the present work (*Claramunt, et al., 2005; Cavallaro, et al., 2009*). The increasing number of dominant missense mutations in the *GDAP1* gene, mainly the p.R120W, undoubtedly shows that some *GDAP1* mutations by themselves cause a mild CMT phenotype.

The mechanism by which a dominantly inherited mutation in the *GDAP1* gene causes disease is largely unknown, although one explanation could be that these dominant mutations could have a negative effect. GDAP1 is a mitochondrial fission protein localized in the mitochondrial outer membrane, functioning as a tail-anchored protein (*Niemann, et al., 2005; Pedrola, et al., 2005; Pedrola, et al., 2008; Wagner, et al., 2009*) that promotes fission without increasing the risk of apoptosis (*Wagner, et al., 2009*). The overexpression of *GDAP1* carrying missense mutations including the dominant p.R120W one, leads to the fragmentation of the mitochondrial network (*Pedrola, et al., 2008*). Different malfunction in mitochondrial dynamics have been postulated according to the mode of inheritance: recessive *GDAP1* mutations seem to lead to a reduction of fission activity whereas dominant *GDAP1* mutations may impair mitochondrial fusion and cause mitochondrial aggregation. This latter mechanism may be similar to some pathogenic *MFN2* mutations (CMT2A) in which

mitochondrial fusion activity is not overly affected, but there is excessive mitochondrial aggregation and impairment of mitochondrial transport (*Baloh, et al., 2007; Detmer and Chan, 2007; Niemann, et al., 2009*). This observation emphasizes that both *GDAP1* and *MFN2* may be involved in the same pathway of axonal CMT pathophysiology, explaining the clinical and neuroimaging similarities.

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TABLE LEGENDS

Table 1: Clinical data of the series.

A: asymptomatic, * : Patients in which only the clinical parameters were used to calculate the CMTNS score (no electrophysiological data available), LL= lower limbs, DF= dorsiflexion, ^{R} = right, ^{L} = left, PF= plantar flexion, TE = toe extension, IHM= intrinsic hand muscles. I= impossible, D= difficulty, N= normal, UL = upper limbs, DTR = deep tendon reflexes, - = absent reflex, + = mildly depressed reflex, ++ = normal reflex.

Table 2: Nerve conductions and MRI data

CMAP= compound muscle action potential, MCV= motor nerve conduction velocity, SCV= sensory conduction velocity, SNAP= compound sensory nerve action potential. NP= not performed; NR= no response, IFM= intrinsic foot muscles, DPC= deep posterior compartment, AC= Anterior compartment, LC= lateral compartment, 0 = no fat signal in muscle, 1 = some fatty streaks, 2 = fat occupying a minor part of muscle, 3 = similar amount of fat and muscle tissue, 4 = fat occupying the greater part of muscle.

FIGURE LEGENDS

Figure 1: Pedigrees of the 4 affected families

Squares = males, circles = females, shaded symbols = affected, dot = affected by history. Family B has been previously reported (*Claramunt, et al., 2005*) and includes two individuals (*) who have not been clinically assessed in this moment.

Figure 2: MRI of the foot and calf muscles in mild and severe phenotypes

A) Axial T1 weighted images showing fatty infiltration in the intrinsic foot muscles from left to right of a control subject, an asymptomatic patient (patient A-II2) and a moderately severe patient (patient C-II2).

B) Axial STIR (above) and T1 weighted (below) images of the calf of patients with a mild phenotype (patient C-I2 & B-II1 respectively). There is muscle edema and fatty infiltration in the superficial posterior compartment of the calf (arrows).

C) Axial T1 weighted images of the calf (left) and thigh (right) of patients with a severe phenotype (patient A-I1 & D-II1 respectively) showing fatty substitution in all the muscle compartments of the calf (posterior > anterolateral) and distal thigh.

~~**Figure 3**: Histograms representing myelinated fibre distribution of patients A-II1 and C-II2 (filled bars). Note the predominant loss of large myelianted fibres compared with a control subject (open bars).~~

Figure 3: Semithin and electron microscope views of the sural nerve biopsy.

A) Semi-thin transverse section of Patient A-II1 showing a pronounced depletion of large myelinated fibres. Note as well thinly myelinated fibres, regenerative clusters and few onion bulb formations.

Plates B to F display distinct electron microscope views. B) Onion bulb formation surrounding a thinly myelinated axon. C) Axonal atrophy with axolemma detachment from myelin sheath. D) Normally myelinated axon with focal accumulates of abnormal mitochondria and paucity of microtubules.

E) Bulb formation encircling a regenerating cluster of unmyelinated axons.

F) Regenerative cluster composed of a bundle of axons, one of them with a tiny myelin sheath, showing axoplasmic degenerative features.

Bar= 10 μm in B, 2 μm in C, D, F, G, 1 μm in E