Supplementary Methods

Electroneurography

Nerve conduction studies were performed using a clinical data acquisition system (Dantec Keypoint, UK). For each test the patient was positioned comfortably on an examination couch in an electrically shielded clinic room and upper/lower limb skin temperature measured to ensure it was within the accepted range for testing (30-36.5 degrees centigrade).

Our standard protocol for the assesment of weakness, including suspected neuropathy or motor neurone disorder is to test:

1. The most involved limb first if mild, but if severe, to test the least involved limb first.
2. At least three limbs (i.e. one upper and one lower) and a third limb for symmetry.
3. Sural and superficial peroneal nerve sensory responses in the lower limbs.
4. Peroneal and tibial CMAPs, MNCVs and F-responses in the lower limbs.
5. Radial, median and ulnar sensory responses in the upper extremity (a distance of 13-14cm between ring stimulating electrode applied to the proximal phalanx of digit and the active recording electrode is used).
6. Median and ulnar CMAPs, MNCVs and F-responses in the upper limb (8cm between distal stimulation site and active ADM/APB recording electrode; below elbow stimulation for ulnar nerve <3cm distal to medial epicondyle; ~10cm distance between below and above elbow median/ulnar nerve stimulation sites).
7. Examine additional nerves if required to determine whether the neuroapthy is axonal or demyelinating, and to look for evidence of more widespread conduction block (defined according to the 2003 AAEM criteria[17]).
8. Where there is a significant reduction in CMAP amplitude at proximal stimulation sites, firstly the stimulus current is increased to the maximum stimulator output (100mA; duration

1ms) to ensure that it is supramaximal and the same process is repeated at more proximal stimulus locations. Secondly, to ensure that the observed diminution in CMAP amplitude cannot be explained by a congenital anomaly, in other words, to exclude the presence of a Martin-Gruber anastomosis (cross-over of median nerve motor fibres to the ulnar nerve at the wrist within the proximal forearm), Type I (median innervates ADM) or Type II (median innervates FDI), the median nerve is stimulated at the wrist and elbow, whilst recording from FDI/ADM; the CMAP from proximal stimulation is increased in amplitude compared with the CMAP resulting from stimulation at the wrist.

The strategy for the first diagnostic electroneurographic examination (2011) was guided by the clinical history of chronic progressive asymmetrical weakness and physical examination findings. The purpose of subsequent electroneurographic examinations (2015 and 2017) was to assess for electrophysiological progression.

SNAP and CMAP amplitudes were measured peak-to-peak. CMAP area was measured from baseline-to-peak. Results of nerve conduction studies were compared with published normative data (see Supplementary Tables 1 & 3; see also Supplementary references).

Needle Electromyography

A concentric needle electrode (TECATM MedelecTM; Carefusion, USA) was used for electromyography (EMG). Needle EMG signals were band-pass filtered at 10Hz-10kHz .

The approach to needle examination was guided by clinical examination and electroneurographic findings, with the following considerations, according to standard EMG protocols:

1. In a symptomatic limb at least three muscles (covering proximal and distal muscles) innervated by different nerves and roots must be examined.

2. If abnormalities are found (e.g. fibrillation potentials, positive sharp waves, fasciculation potentials, MUAP changes, etc.) muscles in each of at least three extremities are examined.

3. If clinically involved muscles show no definite abnormality, extensive time is not expended examining normal muscles.

4. Other muscles are tested if subclinical abnormalities are identifed on nerve conduction studies, for example confirming a distribution of changes consistent with neuropathy or radiculopathy.

5. Any abnormalities are confirmed by examination of at least one contralateral muscle.

6. If ALS suspected muscles in at least 2 extremities plus a cranial nerve (e.g. tongue) and a thoracic paraspinal are examined.

Protein sample preparation and LC-MS/MS studies

Cell lysis and carbamidomethylation

Four replicates of fibroblasts derived from the patient and six replicates of fibroblasts derived from age and gender matched controls were used for our proteomic study. Each replicate contained approximately 1 mg of cells. Snap-frozen cell pellets were used for enrichment of membrane and membrane-associated proteins. More precisely, each sample was lysed in 0.3 mL of 0.1 M NaHCO3 (pH 10.7) buffer containing Complete Mini on ice for 30 min. Afterwards, samples were centrifuged at 68.000 xg for 30 min at 4 °C. The cytosolic fractions and membrane fractions were separated. Membrane fractions were re-suspended in 0.3 mL of 50 mM Tris-HCl (pH 7.8) buffer containing 150 mM NaCl, 1 % SDS, and Complete Mini. Cytosolic fractions underwent ethanol precipitation (5 volumes of 96% ultrapure ethanol; overnight at -20 °C). Next, cytosolic fractions were centrifuged at 18.000 xg for 30 minutes at 4 °C, and pellets containing cytosolic proteins were collected and resuspended in 0.3 mL of 50 mM Tris-HCl (pH 7.8) buffer containing 150 mM NaCl, 1 % SDS, and Complete Mini.
Protein concentrations of cytosolic and membrane fractions of each replicate were determined by BCA assay according to manufacturer's instructions. Then, cysteines of the proteins were reduced by addition of 10 mM DTT at 56°C for 30 min, followed by alkylation of free thiol groups with 30 mM IAA at room temperature (RT) in the dark for 30 min.

Sample preparation and trypsin digestion

Further sample preparation was performed using filter-aided sample preparation (FASP). Briefly, 100 µg of protein extract was 10-fold diluted with freshly prepared 8 M urea/100 mM Tris-HCl (pH 8.5) buffer and placed on a centrifugal device Nanosep 30 KDa Omega (Life Science). For all steps, the device was centrifuged at 13,800 g at RT for 20 min. First, to eliminate residual SDS, three washing steps were carried out with 100 µL of 8 M urea/100

mM Tris-HCl (pH 8.5). Then, for buffer exchange, the device was washed thrice with 100 µL of 50 mM NH_4HCO_3 (pH 7.8). Next, 100 µL of proteolysis buffer comprising of trypsin (Promega) (1:25 w/w, protease to substrate), 0.2 M GuHCl and 2 mM CaCl2 in 50 mM NH_4HCO_3 (pH 7.8), was added to the device and incubated at 37°C for 14 h. Afterwards, the generated tryptic peptides were recovered by centrifugation with 50 µL of 50 mM NH_4HCO_3 followed by 50 µL of ultra-pure water. Finally, peptides were acidified by addition of 10 % TFA (v/v) and digests were quality controlled in a reversed-phase HPLC.

LC-MS/MS analysis

Replicates were measured using a Dionex UltiMate 3000 RSLCnano System coupled to an Orbitrap Elite Mass Spectrometer (both from Thermo Scientific). Briefly, peptides were preconcentrated on a 100 µm x 2 cm C18 trapping column for 10 min using 0.1 % TFA (v/v) at a flow rate of 20 µL/min followed by separation on a 75 µm x 50 cm C18 main column (both from Acclaim Pepmap, Thermo Scientific) with a 174 min LC gradient ranging from 3-45 % of 84 % ACN, 0.1 % FA (v/v) at a flow rate of 250 nL/min. MS survey scans were acquired in the Orbitrap from m/z 300 to 1500 at a resolution of 60,000 using the polysiloxane ion at m/z 371.101236 as lock mass. The ten most intense signals were subjected to collision induced dissociation (CID) in the ion trap, taking into account a dynamic exclusion of 30 s. CID spectra were acquired with a normalized collision energy of 35 % and an activation time of 10 ms. AGC target values were set to 106 for Orbitrap MS and 104 for ion trap MSn scans, and maximum injection times were set to 100 ms for both full MS and MSn scans.

Label free data analysis

Further analysis of the acquired label free quantitative MS data was performed using the Progenesis Qi software from Nonlinear Dynamics (Newcastle upon Tyne, U.K.). For the

cytosolic and the membrane enriched proteomic comparison, four replicates of fibroblast measurements obtained from PTEN patient were compared to six replicates from age and gender matched controls, separately. Alignment of MS raw data was conducted by Progenesis Qi which automatically selected one of the LC-MS files as reference. After peak picking, only features within retention time and m/z windows from 0-115 min and 300-1500 m/z, with charge states +2, +3, and +4 were considered for peptide statistics and analysis of variance (ANOVA). MS/MS spectra were exported in an mgf file as peak lists. The mgf peak lists were searched against a concatenated target/decoy version of the human Uniprot database, (downloaded on 22nd of July 2015, containing 20,273 target sequences) using Mascot 2.4.0 (Matrix Science), X! TANDEM Vengeance (2015.12.15.2) and MS-GF+ Beta (v10282) (12/19/2014) with the help of searchGUI 3.1.4. Trypsin with a maximum of two missed cleavages was selected as enzyme. Carbamidomethylation of cysteine was set as fixed and oxidation of methionine was selected as variable modification. MS and MS/MS tolerances were set to 10 ppm and 0.5 Da, respectively.

We used our PeptideShaker software 1.13.3 (http://code.google.com/p/peptide-shaker/) (Referenz) for interpretation of peptide and protein identifications from searchGUI and Mascot. Combined search results were filtered at a false discovery rate (FDR) of 1 % on the protein level and exported using the advanced PeptideShaker features that allow direct re-import of the quality-controlled data into Progenesis Qi. Peptide sequences containing oxidized methionines were excluded for further analysis. Only proteins that were quantified with unique peptides were exported. Then, for each protein, the average of the normalized abundances (obtained from Progenesis Qi) from the replicate analyses was calculated to determine the ratios between the PTEN patient and the controls. Only proteins which were (i) commonly quantified in all the replicates with (ii) at least two unique peptides, (iii) an ANOVA p-value of <0.05 (Progenesis Qi) and (iv) an average log 2 ratio of which protein

that was either higher than the up-regulated cut-off or lower than the down-regulated cut-off was considered as regulated. The cut-off values were determined based on the 2x standard deviation and the normal distribution from all identified protein's log2 ratio in which the bell curve is symmetric around the mean. Therefore, an average log2 ratio of a protein which < -2.08 or > 2.38 (corresponding to ~5.22-fold regulation; log2 ratios of 1.10) for comparative membrane-associated profile and an average log2 ratio < -3.03 or > 2.81 (corresponding to 7.03-fold regulation; log2 ratios of 0.92) for comparative cytosolic profile were considered as regulated.

Supplementary References

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