Supporting information S1

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1. Supplementary findings: Genetic Profile of the patients

From the genetic testing, 19 patients were found to have new mutation. Three 3 new deletions in SPG4: c.1620_1623delGACT/InsC (3 related patients), exon 16 deletion (MLPA) (1 patient), exon 17 deletion (MLPA) (4 patients) and one deletion in SPG11 c.408_428del21 p.E136_I142del (1 patients). Other 10 mutations were found in 10 SPG4 patients (S1 Table).

2. ROIs delineation for DTI sampling

All delineations of the Regions of Interest (ROIs) were performed by three operators (26, 16 and 3 years of experience, respectively for DM, HH and MV). The ROIs were applied to the DTI maps in 2 different stages: an instruction stage, during which in two weeks of daily work, the accuracy of MRI anatomical location of the ROIs were discussed among the three operators and then reconstructed for all the 22 normal subjects and repeated for the patient group. When the accordance of anatomical positioning and the corresponding FA and MD values were close to both the operator, in the last stage two operators (HH and MV) independently created individual data set, extracting single FA and MD values for each volunteer and patient; inter-observer agreement was also calculated (HH *vs* MV). ROIs positioned by the second operator (MV) were repeated twice in order to study intra-observer agreement.

3. H-MR spectroscopy results

By H-MRS samplings no significant differences between HPS patients and controls were identified. The recent reports on MRS technique concerning some minimal variations in HSP patients [S25-S27] are not confirmed in this study: we found no differences in 1H-MRS metabolic profile between HSP patients and controls and no correlation with clinical/paraclinical variables. Possible explanations could be the limited strength of the magnet (1.5T), the limited dimension of the sampling volume and the nature of the possible metabolic variation itself, which may be too subtle to be detected in a limited number of patients.

4. S1 Supplementary References

S1 Supplementary References

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