Supplemental document

Predominant spastic paraparesis associated with the D178N mutation in *PRNP*

Clinical data

The patient's past medical history included macular degeneration, cataracts, strabismus and type 2 diabetes treated only with diet. During admission electroneurography yielded normal findings except for incidental carpal tunnel syndrome. MRI of the spine demonstrated several vertebrae compressions were found at the thoracal and lumbar levels (Th9, Th11, L1 and L5) but did not compromise the spinal cord. An echocardiography showed mild septal hypertrophy but normal ejection fraction and her brain natriuretic peptide was normal ruling out cardiac failure. Palliative care was provided at home following the wishes of the patient and next-of-kin.

Genetic analyses

Whole-genome sequencing (WGS) was performed. Briefly, genomic DNA was extracted from blood, extracted DNA was converted to sequencing libraries using a PCR-free paired-end protocol (Illumina TruSeq). Sequencing was performed on the Illumina NovaSeq 6000 platforms aiming at 30x median coverage. Data was aligned to GRCh37 and processed with the bioinformatics pipeline MIP v6.0.17. Variants were visualized and reviewed in Scout with an *in-silico* panel consisting of 109 genes, associated with adult lipofuscinosis, neurodegeneration, and motor neuron disease. Included genes were: *ALS2, ANG, ANXA11, APP, AR, ATN1, ATP13A2, ATP1A3, ATXN1, ATXN10, ATXN2, ATXN3, C19orf12, C9orf72, CCNF, CHCHD10, CHMP2B, CLN1, CLN3, CLN5, CLN6, CSF1R, CTSD, CTSF, DAO, DCTN1, DNAJC5, DNAJC6, DNMT1, EPHA4, EPM2A, ERBB4, FBXO7, FIG4, FTL, FUS, GBA, GCH1, GLE1, GRN, HNRNPA1, HNRNPA2B1, HTT, ITM2B, JPH3, LRRK2, LYST, MAPT, MATR3, MME, NEF4, NEFH, NEK1, NHLRC1, NOP56, NOTCH3, OPA3, OPTN, PANK2, PARK7, PFN1, PINK1, PLA2G6, PLD3, PON1, PON2, PON3, PPARGC1A, PPP2R2B, PPT1, PRKN, PRKRA, PRNP, PRPH, PSEN1, PSEN2, RAB39B, SETX, SIGMAR1, SLC30A10, SLC39A14, SLC52A2, SLC52A3, SLC6A3, SNCA, SOD1, SORL1, SPG11, SPR, SQSTM1, SYNJ1, TAF15, TARDBP, TBK1, TBP, TH, TMEM106b, TREM2, TUBA4A, TUBB4A, TYROBP, UBE3A, UBQLN2,*

UNC13A, VAPB, VCP, VPS13A, VPS35, WDR45. Possible C9orf72 repeat expansion was also analyzed from the data and found to be normal. The D178N variant in *PRNP* was confirmed by secondary method at an external genetic laboratory (Blueprint Genetics, Finland). The results of the genetic studies became available soon after the patient had passed away.

Neuropathology

Three minimal infarctions, size less than 2 mm, with gliosis were found in the motor cortex.

Western blot

Western blotting / Sodium phosphotungstic acid (NaPTA) analysis was performed using the SUPERSIGNAL detection system and the development System XRS+ Bio-Rad (e-10). This case was initially PrP negative on a standard diagnostic Western Blot and centrifugal concentration (200ul). NaPTA Western Blot analysis showed detectable levels of the protease-resistant prion protein fragment in the frontal cortex (only tissue available for biochemical analysis). The isoform pattern showed abundance of the di-glycosylated band with a non-glycosylated band with a molecular mass of around 19kDa, suggestive of a "type 2B" isoform. Low levels of PrPres, and PrPres type 2B or 2A/B have previously been observed for FFI (D178N-129M) cases referred to The National CJD Research & Surveillance Unit in the United Kingdom.

Discussion

Of interest, a founder effect for the D178N mutation in *PRNP* has been described in families from the Basque Country (e-9). Another *PRNP* mutation associated with predominant paraparesis is P105L (2)

Additional references

e-1 Llorens F, Zarranz JJ, Fischer A, et al. Fatal Familial Insomnia: Clinical Aspects and Molecular Alterations. Curr Neurol Neurosci Rep. 2017 (4):30.

e-2 Medori R, Tritschler HJ, LeBlanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. N Engl J Med. 1992;326(7):444-9.

e-3 Montagna P, Cortelli P, Avoni P, et al. Clinical features of fatal familial insomnia: phenotypic variability in relation to a polymorphism at codon 129 of the prion protein gene. Brain Pathol. 1998;8(3):515-20.

e-4 Taniwaki Y, Hara H, Doh-Ura K, et al. Familial Creutzfeldt-Jakob disease with D178N-129M mutation of PRNP presenting as cerebellar ataxia without insomnia. J Neurol Neurosurg Psychiatry. 2000;68(3):388.

e-5 Synofzik M, Bauer P, Schöls L. Prion mutation D178N with highly variable disease onset and phenotype. J Neurol Neurosurg Psychiatry. 2009;80(3):345-6.

e-6 Cortelli P, Fabbri M, Calandra-Buonaura G, et al. Gait disorders in fatal familial insomnia. Mov Disord. 2014;29(3):420-4.

e-7 Fulbright RK, C Hoffmann C, Lee H, et al. MR imaging of familial Creutzfeldt-Jakob disease: a blinded and controlled study. AJNR Am J Neuroradiol; 2008;29(9):1638-43.

e-8 Sano K, Satoh K, Atarashi R, et al. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. PLoS One. 2013;8(1): e54915.

e-9 Rodríguez-Martínez A, Barreau C, Coupry I et al. Ancestral origins of the prion protein gene D178N mutation in the Basque Country. Hum Genet. 2005;117(1):61-9.

e-10 Peden AH, Kanguru L, Ritchie DL, Smith C, Molesworth AM. Study protocol for enhanced CJD surveillance in the 65+ years population group in Scotland: an observational neuropathological screening study of banked brain tissue donations for evidence of prion disease. BMJ Open. 2019;9(10):e033744.

Parameter in CSF	**Value in patient III:4**	**Reference value**
Neurofilament light protein	4930*	<1850 ng/L
Tau	615*	<404ng/L
Phospho-tau	48	<56.5 ng/L
β-amyloid 42	532*	>599 ng/L
β-amyloid 42/40	0.6*	>0.68
14-3-3	Absent	<2.0 ng/mL
PrP^{sc} detection (RT-QuIC)	Absent	Absent

Table e-1: Summary of CSF findings in a patient with prominent spastic paraparesis harboring the D178N mutation in *PRNP*. CSV albumin, CSV albumin/serum, CSV kappa free light chains (KFLC) and its IF were normal. RT-QuIC yielded negative results in the CSF. *Indicates abnormal value.