**Altered CSF levels of monoamines in hereditary spastic paraparesis 10 – a case series**

METHODS

**Genetic analyses.**In family A, the *SPAST* and *ATL1* genes were first analyzed (Centogene, Rostock, Germany) in the proband (III:1) without the demonstration of any pathogenic mutations. As a second step, mutations in *REEP1* were ruled out by DNA sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). Targeted analysis of *KIF5A* was thereafter ordered, and the heterozygous mutation c.767A>G (p.Asp256Ser) was identified by DNA sequencing (Centogene, Rostock, Germany), and subsequently in the proband’s mother (II:1) [1].

In family B, a gene panel for autosomal dominant spastic paraparesis was used for the index case (II:1), including the following genes: *SPAST*, *ATL1*, *BSLC2*, *HSDP1*, *KIAA096*, *KIF5A*, *NIPA1*, *REEP1*, *RTN2*, *SLC33A* and *ZFYVE27* (Centogene, Rostock, Germany). The heterozygous variant c.967C>T (p.Arg323Trp) was identified and found to segregate in the proband’s son (III:1) [2]. Using bioinformatics tools this variant could affect splicing in this gene.

**Biochemical analyses.**Cerebrospinal fluid (CSF) concentrations of total tau (t-tau) and phosphorylated tau (p-tau) were measured using commercial INNOTEST ELISA methods (Fujirebio Europé, Ghent, Belgium). The CSF β-amyloid 42/40 (Aβ42/40) ratio was determined using the MSD® Abeta-Triplex Assay (Meso Scale Discovery, Gaithersburg, MD, USA). CSF neurofilament light (NFL) chain levels were measured using an in-house ELISA method (Gaetani, 2018). CSF levels of the monoamine metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were determined by high-performance liquid chromatography with electrochemical detection, and age- and length-corrected values were calculated [4]. All samples were analysed as part of a clinical routine, by board-certified laboratory technicians following strict procedures for batch-bridging, analyses and quality control of individual runs, as previously described [5]. The cut-offs correspond to the reference limits used in clinical routine at the Clinical Neurochemistry Laboratory, Mölndal, Sweden.

**References for e-Methods:**

[1] Reid E, Kloos M, Ashley-Koch A, et al. A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). Am J Hum Genet 2002;71:1189-1194.

[2] Rinaldi F, Bassi MT, Todeschini A, et al. A novel mutation in motor domain of KIF5A associated with an HSP/axonal neuropathy phenotype. J Clin Neuromuscul Dis 2015;16:153-158.

[3] Gaetani L, Höglund K, Parnetti L, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. Alzheimers Res Ther 2018;10:8.

[4] Blennow K, Wallin A, Gottfries CG, Månsson JE, Svennerholm L. Concentration gradients for monoamine metabolites in lumbar cerebrospinal fluid. J Neural Transm Park Dis Dement Sect 1993;5:5-15.

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