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Loss of spatacsin function alters lysosomal lipid clearance leading to upper and lower motor neuron degeneration

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ABSTRACT

Mutations in *SPG11* account for the most common form of autosomal recessive hereditary spastic paraplegia (HSP), characterized by a gait disorder associated with various brain alterations. Mutations in the same gene are also responsible for rare forms of Charcot-Marie-Tooth (CMT) disease and progressive juvenile-onset amyotrophic lateral sclerosis (ALS). To elucidate the physiopathological mechanisms underlying these human pathologies, we disrupted the *Spg11* gene in mice by inserting stop codons in exon 32, mimicking the most frequent mutations found in patients. The *Spg11* knockout mouse developed early-onset motor impairment and cognitive deficits. These behavioral deficits were associated with progressive brain atrophy with the loss of neurons in the primary motor cortex, cerebellum and hippocampus, as well as with accumulation of dystrophic axons in the corticospinal tract. Spinal motor neurons also degenerated and this was accompanied by fragmentation of neuromuscular junctions and muscle atrophy. This new *Spg11* knockout mouse therefore recapitulates the full range of symptoms associated with *SPG11* mutations observed in HSP, ALS and CMT patients. Examination of the cellular alterations observed in this model suggests that the loss of spatacsin leads to the accumulation of lipids in lysosomes by perturbing their clearance from these organelles. Altogether, our results link lysosomal dysfunction and lipid metabolism to neurodegeneration and pinpoint a critical role of spatacsin in lipid turnover.

1. Introduction

Hereditary spastic paraplegias (HSP) constitute the second most common group of motor neuron diseases. They are characterized by progressive bilateral weakness, spasticity and the loss of vibration sensation in the lower limbs. These symptoms are mostly due to the degeneration of upper motor neuron axons in the corticospinal tracts (Harding, 1983). The main causes of autosomal recessive HSP are mutations in the *SPG11* gene. Symptoms of SPG11 patients generally appear during the first decade of life and, in addition to spastic gait disorder, include cognitive impairment, mental retardation, peripheral neuropathy, cerebellar ataxia, parkinsonism and retinal degeneration (Anheim et al., 2009; Puech et al., 2011; Stevanin et al., 2008). These symptoms are often associated with progressive thinning of the *corpus callosum* and white matter hyperintensities by brain magnetic resonance imaging (MRI) (Hehr et al., 2007; Stevanin et al., 2007). Mutations in *SPG11* also account for autosomal-recessive Charcot Marie Tooth (CMT) disease (Montecchiani et al., 2016) and slowly progressive juvenile-onset autosomal recessive amyotrophic lateral sclerosis (ALS) without cognitive impairment or abnormal MRI (Daoud et al., 2012; Orlacchio et al., 2010). In agreement, a recent neuropathological analysis of the brain from two patients with the full-blown SPG11 phenotype showed that the pathology partially mimics ALS lesions (Denora et al., 2016).

SPG11 encodes a 2443-amino acid protein called spatacsin. Regardless of the associated phenotype, the vast majority of mutations

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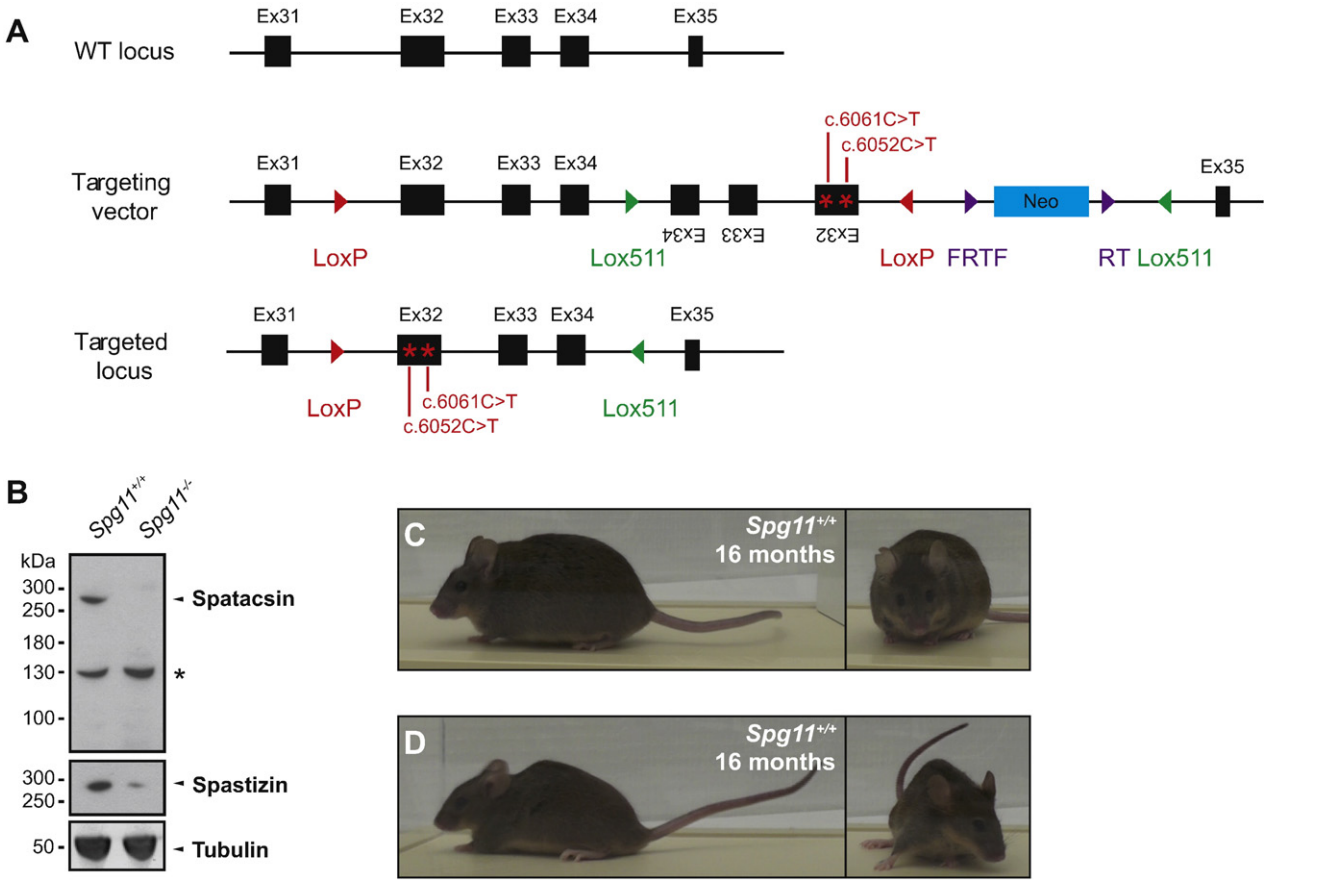

Fig. 1. *Spg11* knockout causes a progressive motor deficit in mice. (A). Diagram showing the genomic structure of the *Spg11* gene (top), the targeting vector (middle) and the targeted locus upon excision of the neomycin resistance cassette and action of the Cre-recombinase (bottom). The mutations introduced were c.6052C>T (p.Arg2018*), corresponding to c.6091C>T (p.Arg2031*) in human and c.6061C>T (p.Gln2021*), corresponding to c.6100C>T (p.Arg2034*) in human. (B) Western blot of brain protein extracts showing the absence of spatacsin in $Spg11^{-/-}$ samples and the strong decrease in the levels of spastizin. Representative image of three independent experiments. * : non-specific band. (C and D), Pictures showing an $Spg11^{+/+}$ (C) and $Spg11^{-/-}$ mouse (D) at 16 months of age. Knockout mice had an abnormal posture and kyphosis of the spine.

identified in SPG11 patients are nonsense or frameshift mutations, which are predicted to lead to the loss of function of spatacsin (Montecchiani et al., 2016; Orlacchio et al., 2010; Stevanin et al., 2008). The nature of the mutation is not responsible for the variability in phenotype as intrafamilial phenotypic variability has been observed, with the same mutations leading to juvenile ALS in one patient and HSP with a thin corpus callosum in his sibling (Daoud et al., 2012).

Spatacsin interacts with proteins involved in membrane-trafficking (Hirst et al., 2013), among which AP5Z1 (SPG48) and spastizin/zFYVE26 (SPG15) are also implicated in hereditary spastic paraplegia, when mutated (Hanein et al., 2008; Slabicki et al., 2010). The symptoms of patients mutated in the *SPG15* or *SPG11* gene are very similar (Hanein et al., 2008), in agreement with their involvement in the same cellular mechanisms. Indeed, recent investigations in fibroblasts of SPG11 and SPG15 patients (Renvoise et al., 2014) and studies in mouse models have suggested that the loss of spatacsin or spastizin alters the function of the endolysosomal system (Khundadze et al., 2013; Varga et al., 2015). Furthermore, spatacsin and spastizin have been shown to be involved in lysosome-recycling after fusion with autophagosomes in HeLa cells (Chang et al., 2014). In agreement, an *Spg11* knockout mouse model, obtained by inserting a genetrap cassette in the first intron of the *Spg11* gene, showed that the loss of spatacsin impairs autolysosome reformation, ultimately leading to depletion of lysosomes and impaired autophagic clearance (Varga et al., 2015). This Spg11 model reproduced the neurodegeneration in the motor cortex and cerebellum observed in SPG11 patients, but did not show any early-onset motor or cognitive deficits, amyotrophy or alterations of the *corpus callosum* as frequently observed in SPG11 patients.

We therefore extensively characterized a new *Spg11* knockout mouse model. These $Spg11^{-/-}$ animals displayed early-onset motor

Fig. 2. *Spg11* knockout mice develop a progressive spastic and ataxic gait disorder. (A and B) Gait angle sketch (A) and values recorded (B) during a forced walk on a treadmill. The gait angle decreased in $Spg11^{-/-}$ mice from four months of age ($n \geq 12$ animals/genotype/age; Kruskal-Wallis test; $^{*}p \leq 0.05$ and $^{***}p \leq 0.001$). (C) Step sequence regularity values recorded during a forced walk on a treadmill. $Spg11^{-/-}$ mice exhibited motor coordination impairment from eight months of age ($n \geq 12$ animals/genotype/age; Kruskal-Wallis test; $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$). (D and E) Rotarod duration (D) and maximum speed (E). The duration and maximum speed of $Spg11^{-/-}$ in accelerated rotarod testing was less than those of heterozygous and control mice at as early as six weeks of age ($n \geq 12$ animals/genotype/age; Kruskal-Wallis test; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$). (F and G) The Y-maze principle (F) and spontaneous alternation values (G). Knockout mice did not show a preference between the visited and the unknown arm of the maze from four months of age ($n \geq 12$ animals/genotype/age; Kruskal-Wallis test; $^{***}p \leq 0.001$). This altered cognitive behavior underlines a spatial learning or a memory deficit. (H and I) Fear conditioning. Percentage of time spent in a frozen posture on day 1 before and after electrical shocks (H). There was no obvious learning deficit during the conditioning of the mice at any age. Percentage of time spent in a frozen posture on day 2, without any electrical shocks, after conditioning (I). Although the task was learned, knockout mice spent less time in a frozen position from eight months of age, indicating a cognitive and memory deficit ($n \geq 10$ animals/genotype/age; Kruskal-Wallis test; $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$).