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Expanding the Spectrum of AP5Z1-Related Hereditary Spastic Paraplegia (HSP-SPG48): A Multicenter Study on a Rare Disease

Biallelic mutations in AP5Z1 are known to cause a rare, autosomal-recessive, complex form of hereditary spastic paraplegia (HSP) referred to as SPG48 (MIM#613647). 1 To date, only 11 SPG48 patients have been reported. The clinical spectrum of SPG48 is complex and heterogeneous, presenting with neuropathy, ataxia, dystonia, and parkinsonism in addition to spastic paraplegia (SP). AP5Z1 codes for the subunit of the AP-5 complex, implicated in vesicular-mediated intracellular sorting and trafficking of cargo proteins. 1 - Functional studies demonstrate the accumulation of multilamellar structures (endolysosomes) in SPG48 skin fibroblasts. 2

Here, we screened 2035 HSP patients from 3 tertiary centers (Athens, University of Athens [UOA]; London, University College London; and Paris, Paris, Sciences & Lettres [PSL], Assistance Publique-Hôpitaux de Paris [APHP]) for mutations in AP5Z1 and performed functional studies in 2 cases with pathogenic variants in AP5Z1. We also present a literature review for AP5Z1 cases, a pathway analysis, and follow-up data on previously reported patients, where available (Supplementary Material 1, 4, and 5).

In total, 9 patients from 8 unrelated families carrying biallelic pathogenic variants in AP5Z1 were identified (Fig. 1; Supplementary Material 1). We show that AP5Z1-related disease usually presents with a combination of late-onset SP (mean: 54.3 ± 5.3 years) and axonal neuropathy. Other frequent clinical features in our cohort were urinary incontinence, hearing loss, and visual impairment. Interestingly, 1 patient had epileptic seizures. Brain magnetic resonance imaging (MRI) was available for 6 patients. Leukoencephalopathy and thinning of the corpus callosum (TCC) were present in 1 patient and “ears of the lynx” sign, a “moth-eaten” appearance of the basal ganglia, and TCC in another (Fig. 2). The remaining 4 patients had normal MRI.

Our extended analysis of all SPG48 patients identified in the literature (Supplementary Material 1) shows that 22 AP5Z1 variants are linked to SPG48 worldwide, including...
the 8 newly reported here. Follow-up data were obtained from 3 SPG48 patients, all of whom had a disease duration of ≥10 years. The progression of the disease was slow (Supplementary Material 5), with the phenotype consisting of predominantly severe lower-limb spasticity with patients becoming wheelchair-bound after 10 years from onset.

AP5Z1 protein levels in patient fibroblasts were reduced to undetectable levels, correlating with an accumulation of lysosomal-associated membrane protein 1 (LAMP1)-positive structures and a 25% deficit in recycling between endosomes and Golgi (Fig. 3, Fig. 4, Supplementary Material 6). This was also supported by functional enrichment analyses which pinpointed that within the network of HSP-associated genes mediating membrane trafficking, AP5Z1 relates to endosomal trafficking, determining the fate of sorting endosomes toward lysosomal fusion, the plasma membrane, or the trans-Golgi network (Supplementary Material 4).

In this study we expanded the phenotypic and genotypic spectrum of SPG48 showing that SPG48 is a slowly progressing, late-onset, complicated HSP manifesting with SP,
axonopathy, cognitive impairment in line with the SPG48 patients reported so far [and in Refs 1 and 3] and, interestingly, epileptic seizures (patient G, Supplementary Material 3). Epileptic seizures have not been previously reported in SPG48; however, they are well described in other HSP subtypes, such as SPG11 and SPG15, which are functionally related to SPG48 and in many lysosomal storage diseases. Indeed, our functional studies on SPG48 cell lines confirm defects in endosome and lysosome homeostasis. We also confirm here previously described neuroimaging findings (“ears of the lynx” sign, TCC, and white matter lesions) in a subgroup of patients.

To date, no specific therapies are approved for HSP. Of note, treatment strategies are proposed in complex forms of HSP such as cholesterol-lowering agents for HSP-CYP7B1 (SPG5A), as CYP7B1 gene is involved in the degradation of cholesterol into primary bile acids. A randomized-controlled trial showed that atorvastatin treatment can effectively lower 27-hydroxycholesterol levels in the serum of SPG5 patients, and evolocumab (PCSK9 inhibitor) is currently evaluated in a phase 2 clinical trial (NCT04101643). In addition, “tideglusib” (GSK3β inhibitor) was tested on iPS neuronal lines of an SPG11 patient and decreased cell death.
Our study strengthens the evidence supporting autophagic dysfunction as one of the underlying molecular pathways in HSP\(^7\) and further expands the phenotypic spectrum of AP5Z1-related SPG48.

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**FIG. 4.** Functional studies on SPG48 patient fibroblasts. (A) Immunofluorescence microscopy of patient-derived fibroblasts and treated with monensin for 90 minutes followed by washout for 2.25 hours. In APSZ1-deficient patient lines, there is reduced retrieval of GOLIM4 back to the juxtanuclear region (where GM130 is located) compared to healthy control patient lines. Scale bar: 20 μm.; (B) Quantification of the retrieval defect (the reduction in the level of colocalization of GOLIM4 and GM130) was performed for 2 independent SPG48 patient-derived fibroblast lines using Pearson’s correlation coefficient. At least 20 cells were quantified per condition. Data show mean of 3 independent experiments and results of a 2-tailed Mann-Whitney U test: **\(P < 0.01\). [Color figure can be viewed at wileyonlinelibrary.com]
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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

PRKRA-Related Disorders:
Bilateral Striatal Degeneration in Addition to DYT16 Spectrum

Biallelic mutations on PRKRA cause a rare form of progressive generalized dystonia with bulbar involvement and parkinsonism (DYT16). So far, 32 PRKRA mutated patients have been reported, and brain magnetic resonance imaging (MRI) was normal in all cases except one boy who presented with bilateral striatal degeneration (BSD). We recently observed 2 nonrelated patients who presented at 30 (number 1) and 14 (number 2) months of life, with recurrent fever-induced episodes of acute encephalopathy resulting in cognitive impairment, axial hypotonia, limb spasticity, generalized dystonia, hypomimia, and hypokinesia. MRI revealed BSD in both (Fig. 1), associated to cerebellar atrophy in one.

Whole-exome sequencing detected biallelic PRKRA variants in patient 1: the known mutation P222L and the novel variant G43S were considered likely pathogenic according to ACMG guidelines. Patient 2 had the known C213F variant.

[Corrections added on 12 March 2021 after first online publication: Correspondence address updated for Belen Perez-Dueñas.]

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