

Imaging-based methods to identify prognostic and predictive biomarkers for Hereditary Spastic Paraplegia

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Background

Hereditary spastic paraplegias (HSPs) are rare motor neuron diseases characterized by axonal degeneration involving the lateral corticospinal tracts. The most common is caused by haploinsufficient mutations in the SPG4 gene, which encodes spastin, a microtubule (MT) severing ATPase, that controls cytokinesis, endosomal traffic, lipid droplets (LDs) homeostasis, and axonal transport. Clinically, HSP-SPG4 age of onset and the severity of symptoms are strongly variable even among individuals belonging to the same family. Given this heterogeneity, it is important to identify prognostic biomarkers. To date, no effective disease-modifying therapies are currently available, but approaches based on drugs counteracting dysfunctional mechanisms or spastin-elevating treatments are emerging, so it will be crucial to identify biomarkers that can help to monitor the effects of spastin recovery treatments.

We have developed an automated, simple, rapid, and non-invasive cell imaging-based method to quantify the organization of the MT-cytoskeleton. By using this method, we demonstrated that the dcnc-parameter, measuring the distance between cell and nucleus centroids, is able to distinguish HSP-SPG4 from healthy donor (HD) lymphoblastoid cell lines (LCLs) and peripheral blood mononuclear cells (PBMCs).

We are now extending the dcnc-based method to a larger cohort of SPG4 patient cells to evaluate its sensitivity and specificity in relation with molecular and clinical patient features and to detect the effects of different spastin-elevating drugs. Additionally, we are also focusing on other subcellular components affected by spastin mutations, such as LDs.

Methods

The study included 13 HDs and 18 SPG4 patients with different types of mutations (12 truncating mutations and 6 missense mutations). Cell image analysis was performed using PBMCs or LCLs stained with specific antibodies (anti- β -tubulin-cy3 for MT-cytoskeleton) and dyes (Bodipy™ 493/503 for LD and Hoechst for Nucleus). Automated image acquisition was achieved by using the inverted Nikon Eclipse-Ti microscope and the JOBS module of NisElements 5.11 software to obtain a file containing more than 50 images and 500 cells related to each single sample. The acquired images were then analyzed using Cell Profiler 4.3 software, with an ad hoc designed pipeline that recognizes cellular compartments using thresholding segmentation and measures several parameters, including "dcnc" and "number of LD in each cell (nLD)".

Results

We performed correlation analysis between the "dcnc" parameter and molecular patient features, showing that SPG4 cells from patients with missense mutations have higher "dcnc" than those with truncating mutations. Correlations with clinical features, such as the age at onset of the disease or the Spastic Paraplegia Rating Scale (SPRS) score, are ongoing.

By assessing the effect of three different spastin-elevating drugs, we observed that dcnc-based method is able to detect the reduction of MT defects induced by spastin recovery in cells from SPG4 patients carrying truncating mutations. Similar analyses on cells carrying missense mutations are ongoing.

We have implemented our pipeline to evaluate other cellular components, such as LD, so it automatically recognises the staining of the nucleus, cytoskeleton and LD, in our cells. By using this tool, we have analysed the parameter “nLD”. Correlation analysis between dcnc and “nLD” was performed, showing a positive correlation in SPG4 patients.

Conclusions and future perspectives

Our analyses revealed that the dcnc-based method is able to sense the effects of spastin elevating drugs in cells from SPG4 patients carrying truncating mutations, suggesting a predictive role. Now, we are performing correlation analyses among molecular and clinical patients features and the “dcnc” and “nLD” parameters to evaluate whether our method might have prognostic value. These results will open the possibility to identify new prognostic and predictive tools for HSP-SPG4.

Keywords:

HSP-SPG4
biomarker
neurodegenerative disease

Reference:

- Sardina F, Valente D, Fattorini G, Cioffi E, Zanna GD, Tessa A, Trisciuglio D, Soddu S, Santorelli FM, Casali C, Rinaldo C. New cellular imaging-based method to distinguish the SPG4 subtype of hereditary spastic paraplegia. *Eur J Neurol*. 2023 Jun;30(6):1734-1744. doi: 10.1111/ene.15756. Epub 2023 Mar 26. PMID: 36815539.
- Francesca Sardina, Claudia Carsetti, Ludovica Giorgini, Gaia Fattorini, Gianluca Cestra, Cinzia Rinaldo, Cul-4 inhibition rescues spastin levels and reduces defects in hereditary spastic paraplegia models, *Brain*, 2024;, awae095, <https://doi.org/10.1093/brain/awae095>