sensory examination, and cognitive functions were normal. The patient was able to walk without support but with extensor trunk posture. Brain and cervical spine magnetic resonance imaging was normal.

To identify the disease-causing gene, we carried out high-density single-nucleotide polymorphism genome-wide genotyping in all DNA samples available from the family and whole-exome sequencing (WES) in the patient. We ran linkage analysis assuming an autosomal recessive mode of inheritance and parental consanguinity, which yielded a list of candidate genomic regions (Supporting Information Appendix S1). By inspecting the WES data of the patient for rare homozygous variants with predicted coding or splicing effect (Supporting Information Appendix S1), we identified a 70-nucleotide duplication in exon 2 of *AOPEP* (NM_001193329.1), leading to premature termination in the encoded protein: c.333_402dup (p.Gly135*). This variant is absent in gnomAD.[3] Agarose gel electrophoresis (Fig. 1B), as well as Sanger sequencing (Fig. 1C), confirmed the variant and showed its presence in homozygous state in the affected subject but in none of his unaffected relatives. Furthermore, WES analysis demonstrated no definitive disease-causing variants in other known dystonia genes, nor compelling variants in other genes in the candidate genomic regions (Supporting Information Appendix S1). We therefore consider the novel LOF *AOPEP* variant (Fig. 1D) as disease causing in this patient.

The recently described cases presented with progressive dystonia, predominantly involving upper and lower limbs, with variable involvement of craniocervical and truncal districts.[2] The age at onset ranged from childhood to early adulthood. In three of the four families reported, dystonia was isolated. Our patient also manifested dystonia in the upper limbs in early adulthood, which progressed to the craniocervical and truncal segments.

This work provides further, independent evidence for the involvement of *AOPEP* in early-onset dystonia. Future clinical studies will contribute to better delineating the phenotypic spectrum of *AOPEP*-related dystonia, while functional work is warranted to provide insights into the mechanisms by which *AOPEP* LOF leads to dystonia.

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Data Availability Statement

The data that support the findings of this study are available from the authors upon reasonable request.

Christina Fevga, MD, MSc,[1] Federico Ferraro, MSc,[1] Guido J. Breedveld, BSc,[1] Charulata Savant Sankhla, MD,[2] and Vincenzo Bonifati, MD, PhD[1*]

[1]*Department of Clinical Genetics, University Medical Center, Erasmus MC, Rotterdam, the Netherlands, and* [2]*Department of Neurology, P D Hinduja National Hospital, Mumbai, India*

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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.

Cerebellar and Midbrain Lysosomal Enzyme Deficiency in Isolated Dystonia

The majority of dystonia cases remain of unknown cause even after exhaustive routine diagnostics. Based on the occasional clinical observation of decreased levels of lysosomal enzyme activity in peripheral blood in a relevant proportion of dystonia patients, we measured glucocerebrosidase (GCase) and beta-galactosidase (b-Gal) in postmortem brain tissue of age-, sex-, and post-mortem delay-matched patients and controls from the Queen Square Brain Bank and report reduced lysosomal enzyme activity in the cerebellar dentate gyrus and the superior colliculus (SCol) in dystonia patients (Fig. 1).

The finding that GCase activity was affected in the cerebellar dentate nucleus—the primary cerebellar efferent structure—but not cerebellar cortex (CRB), adds to the current understanding of the role of cerebellar structures in dystonia.[1] The observed activity changes in b-GAL similarly

***Correspondence to:** Dr. Sebastian R. Schreglmann, FEBN, Department of Neurology, University Hospital Würzburg, 97080 Würzburg, Germany; E-mail: skgtsrs@ucl.ac.uk

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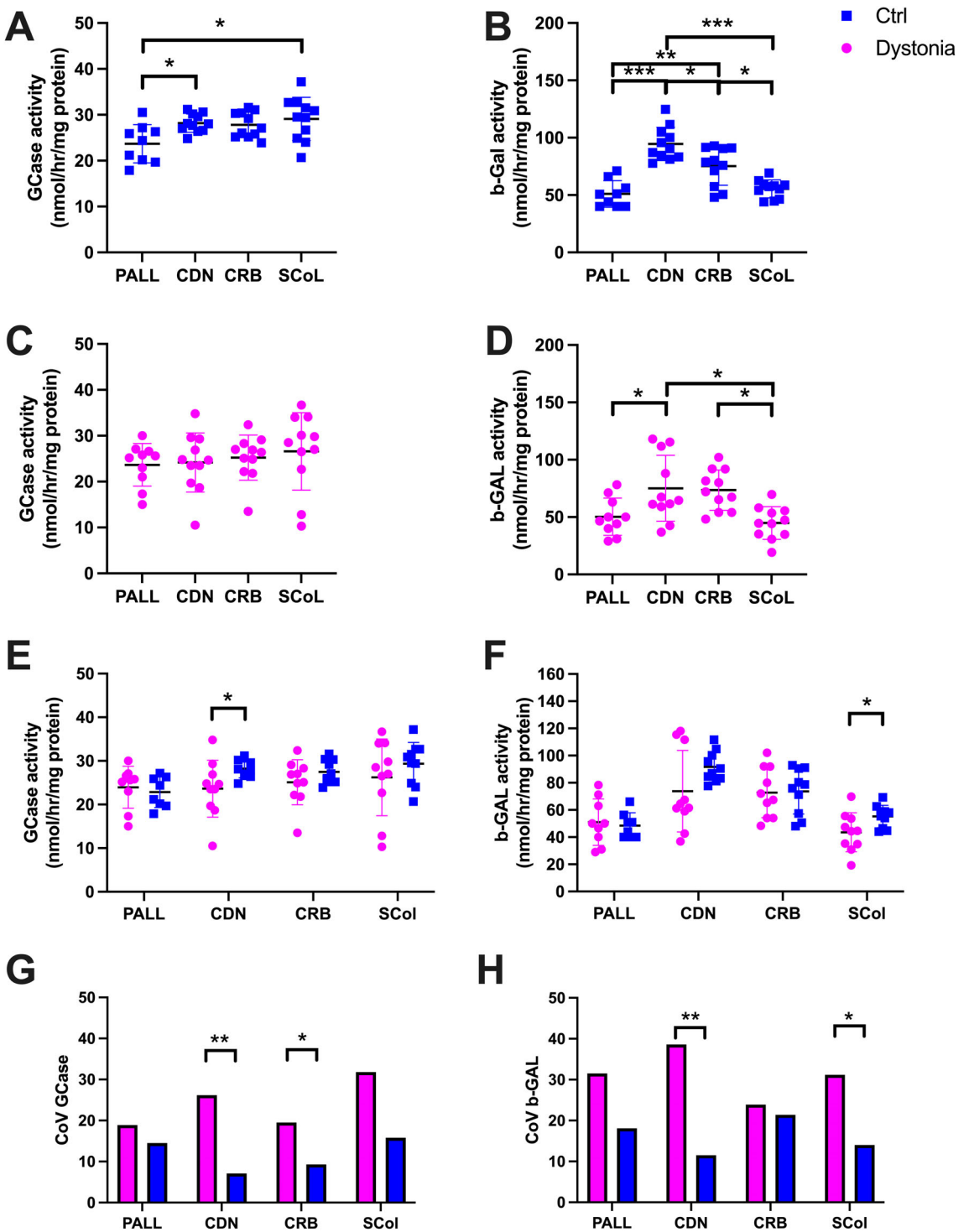

FIG. 1. Lysosomal enzyme activity in postmortem brain region samples: Between brain regions in healthy controls (blue squares), GCase activity was highest in ScoL and lowest in PALL (one-way analysis of variance; $F_{3,38} = 4.336$; $P < 0.01$), whereas b-GAL activity was highest in CDN > CRB > CRB/PALL ($F_{3,38} = 24.36$; $P < 0.0001$; **A**, **B**). This region-specific pattern was lost in dystonia tissue samples (magenta circles) for GCase ($P = 0.72$) and attenuated for b-GAL ($F_{3,39} = 6.52$; $P = 0.001$; **C**, **D**). Mean activity levels for GCase were lower in dystonia versus Ctrl tissue samples in CDN (independent sample t test; $t_{18} = 2.12$; $P = 0.048$) and b-GAL in ScoL ($t_{18} = 2.23$; $P = 0.038$) but did not reach statistical significance in other brain regions (**E**, **F**). Similarly, the CoV—describing the degree of dispersion of measurements per group—was significantly larger among dystonia samples for GCase activity in CDN ($P = 0.0008$) and CRB ($P = 0.03$) with a trend in Scol (P=0.056), as well as for b-GAL in CDN ($P = 0.0023$) and ScoL ($P = 0.03$; **G**, **H**). Abbreviations: glucocerebrosidase (GCase), beta-galactosidase (b-GAL), pallidum (PALL), cerebellar dentate nucleus (CDN), cerebellar cortex (CRB), superior colliculus (SCoL); *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. [Color figure can be viewed at wileyonlinelibrary.com]

document primarily affected cerebellar efferents, but also the dorsal midbrain, lending additional metabolic support to functional imaging data, suggesting changes in the SCol in dystonia.[2] Various lines of evidence ranging from animal studies reporting improvements in lesions, gene expression experiments in monogenic forms, human structural and functional imaging, and eye-blink classical condition experiments point toward an involvement of efferent cerebellar structures in dystonia pathophysiology.[1,3] Thus far, it proved difficult to conclude regarding how far cerebellar activity in dystonia is causal, contributory, or compensatory.[3] In contrast to increased metabolic activity on cerebellar glucose-positron emission tomography imaging, which can be interpreted as both possibly causative and compensatory,[1] our observation of decreased enzyme activity is compatible with a primary deficit within the cerebellar outflow tract.

The b-GAL results overall argue against a purely GCase-mediated effect but more likely general lysosomal activity changes in dystonia. Larger genetic studies are planned to elucidate whether lysosomal dysfunction in dystonia is associated with a specific gene, such as GBA, or broader mechanisms regulating lysosomal function. Mechanistically, endosomal-lysosomal deficiency has recently been reported to be implicated in dystonia due to mutations affecting the homotypic fusion and vacuole protein sorting complex, postulating disrupted cellular processes in motor control networks as a possible mechanism.[4] Similarly, network signaling abnormalities[5] and synaptic dysfunction[6] have been described in the context of lysosomal storage disorders, and future studies should explore if they provide a possible mechanistic relation between lysosomal and network dysfunction in dystonia.

Brain regions in this study were chosen based on their presumed role in dystonia pathophysiology[1,2,7] and tissue availability and thus are not representative. We acknowledge the limitations regarding phenotypical information and statistical power due to the paucity of dystonia brain donors (Table S1). The presence of signs of pathological aging in some donors, reflecting the age at death, was balanced between groups and unlikely to have affected results. Medication-related bias seems equally unlikely, especially for botulinum toxin injections, the most frequently used medication in our sample.

In summary, our observations provide preliminary evidence for a possible role of lysosomal dysfunction in isolated dystonia. Although the enzyme activity pattern identified points to a primary role of cerebellar output-/brainstem structures, the exact mechanism of how lysosomal dysfunction causes dystonia remains to be established.

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Sebastian R. Schreglmann, MD, PhD,[1,2*] Derek Burke, MSc, PhD,[3] Amit Batla, MD, DM,[1] Nikola Kresojevic, MD, PhD,[4] Nicholas Wood, MD, PhD,[1] Simon Heales, PhD, FRCPath,[3,5] and Kailash P. Bhatia, MD, FRCP[1]

[1]*Department of Clinical and Movement Neurosciences, Institute of Neurology, London, United Kingdom,* [2]*Department of Neurology, University Hospital Würzburg, Würzburg, Germany,* [3]*Enzyme Unit, Great Ormond Street Hospital, London, United Kingdom,* [4]*Neurology Clinic, University Clinical Centre of Serbia, Medical Faculty, University of Belgrade, Belgrade, Serbia, and* [5]*UCL BRC Great Ormond Street Institute of Child Health, London, United Kingdom*

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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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.

In Vivo Brain Sodium Disequilibrium in *ATP1A3*-Related Rapid-Onset Dystonia-Parkinsonism

ATP1A3-related neurological disorders display a broad clinical spectrum with three predominant phenotypes, including rapid-onset dystonia-parkinsonism (RDP).[1] The *ATP1A3* gene encoding the α-subunit (subtype 3) of the Na^+/K^+-ATPase enzyme maintains the neuronal electrochemical gradient by removing intracellular sodium in exchange for extracellular