AOPEP Homozygous Loss-of-Function Variant in an Indian Patient with Early-Onset Generalized Dystonia

Biallelic loss-of-function (LOF) variants in the *AOPEP* gene were recently identified in five European patients with generalized or multifocal dystonia.[1,2] In this letter, we present an Indian case with early-onset, generalized dystonia carrying a novel homozygous LOF *AOPEP* variant.

A 31-year-old man born to consanguineous parents of Indian ancestry (Fig. 1A, II-1) presented with complaints of involuntary posturing of the neck, trunk, and both upper limbs since 20 years of age. His symptoms started after an injury resulting in the fracture of his right thumb. Within a month after the injury, he developed abnormal posturing of the right hand. After a few months, abnormal posturing involved both upper limbs, and subsequently the neck and the trunk. He later experienced changes in his voice and difficulty in uttering words and swallowing. His family history was negative for neurological disorders. Perinatal history and medical history were unremarkable.

Upon examination, we found marked dysarthria, laryngeal dysphonia, kyphoscoliosis, retrocollis, and truncal and bilateral hand dystonia (Supporting Information Supplementary Video S1). Hypertonia was evident in both upper and lower limbs. Assessment of deep tendon reflexes and limb muscle

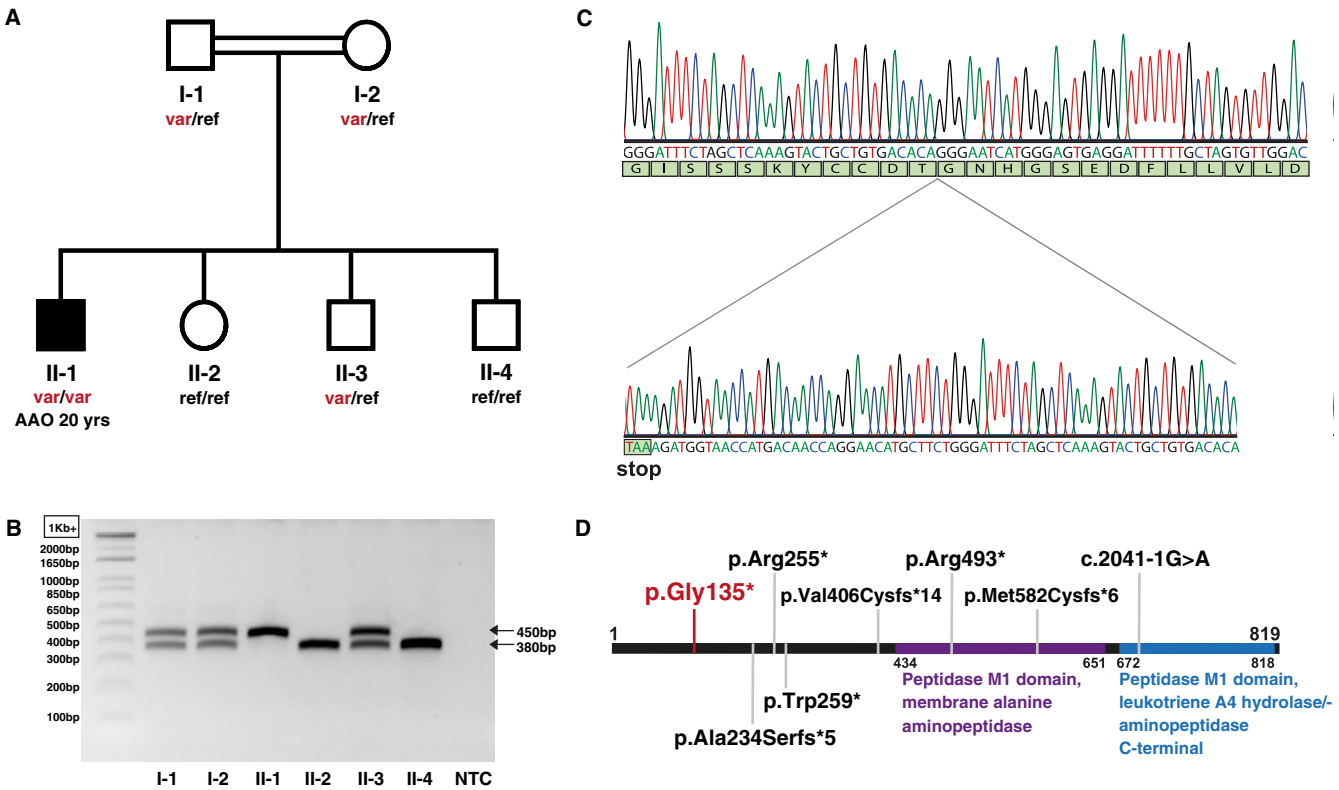

FIG. 1. Pedigree and genetic results. (**A**) The *AOPEP* variant is present in homozygous state in the subject affected by dystonia (black symbol) but in none of his unaffected relatives (white symbols). (**B**) Agarose gel electrophoresis of PCR fragments containing the *AOPEP* exon 2; wild-type allele: 380 bp; variant allele: 450 bp. (**C**) Electropherogram showing the 70-nucleotide duplication in *AOPEP* exon 2 in the DNA amplified from the affected subject. (**D**) Schematic representation of the AOPEP protein with known functional domains[4] and variants reported in patients with dystonia in recent studies (gray anchors) and in this study (in red). AAO, age at onset; NTC, negative control; ref, reference (wild-type) allele; var, *AOPEP* c.333_402dup (p.Gly135*) variant allele. [Color figure can be viewed at wileyonlinelibrary.com]

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strength as well as cerebellar examination, eye movements, 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.

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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

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