





## RESEARCH ARTICLE

# Biallelic *AOPEP* Loss-of-Function Variants Cause Progressive Dystonia with Prominent Limb Involvement



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**ABSTRACT: Background:** Monogenic causes of isolated dystonia are heterogeneous. Assembling cohorts of affected individuals sufficiently large to establish new gene–disease relationships can be challenging.

**Objective:** We sought to expand the catalogue of monogenic etiologies for isolated dystonia.

**Methods:** After the discovery of a candidate variant in a multicenter exome-sequenced cohort of affected individuals with dystonia, we queried online platforms and genomic data repositories worldwide to identify subjects with matching genotypic profiles.

**Results:** Seven different biallelic loss-of-function variants in *AOPEP* were detected in five probands from four unrelated families with strongly overlapping phenotypes. In one proband, we observed a homozygous nonsense variant (c.1477C>T [p.Arg493\*]). A second proband harbored compound heterozygous nonsense variants (c.763C>T [p.Arg255\*]; c.777G>A [p.Trp259\*]), whereas a third proband possessed a frameshift variant (c.696\_697delAG [p.Ala234Serfs\*5]) in *trans* with a splice-disrupting alteration (c.2041-1G>A). Two probands (siblings) from a fourth family shared compound heterozygous frameshift alleles (c.1215delT [p.Val406Cysfs\*14]; c.1744delA

[p.Met582Cysfs\*6]). All variants were rare and expected to result in truncated proteins devoid of functionally important amino acid sequence. *AOPEP*, widely expressed in developing and adult human brain, encodes a zinc-dependent aminopeptidase, a member of a class of proteolytic enzymes implicated in synaptogenesis and neural maintenance. The probands presented with disabling progressive dystonia predominantly affecting upper and lower extremities, with variable involvement of craniocervical muscles. Dystonia was unaccompanied by any additional symptoms in three families, whereas the fourth family presented co-occurring late-onset parkinsonism.

**Conclusions:** Our findings suggest a likely causative role of predicted inactivating biallelic *AOPEP* variants in cases of autosomal recessive dystonia. Additional studies are warranted to understand the pathophysiology associated with loss-of-function variation in *AOPEP*. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

**Key Words:** *AOPEP*; monogenic dystonia; genomic analysis; loss-of-function variants; rare disease

Isolated dystonia refers to a group of chronic debilitating diseases in which involuntary twisting movements and postural abnormalities arise in the absence of any accompanying movement disorder symptomatology.<sup>1</sup> Although isolated dystonia can coexist with non-movement disorder-related neurological features in multiple phenotypically complex conditions, the most common forms seen in adult neurology practices are pure clinical presentations, varying broadly in age of onset and anatomic distribution.<sup>2</sup> With the widespread use of comprehensive genomic analysis techniques, progress has been made in the elucidation of monogenic causes of isolated dystonia.<sup>3</sup> Nevertheless, 85%–95% of affected individuals remain without an etiologic diagnosis after the application of whole-exome or whole-genome sequencing.<sup>4,5</sup> In recent years, Mendelian disease gene discovery has been substantially enhanced by international collaborative matchmaking efforts and pooling of cases with variants in previously undescribed disease-related genes.<sup>6</sup>

In this work, we used large-scale genomic data mining to identify five individuals from four families with dystonia who harbored biallelic homozygous or compound heterozygous loss-of-function variants in *AOPEP* (also known as *C9orf3*). We show that *AOPEP*-mutated

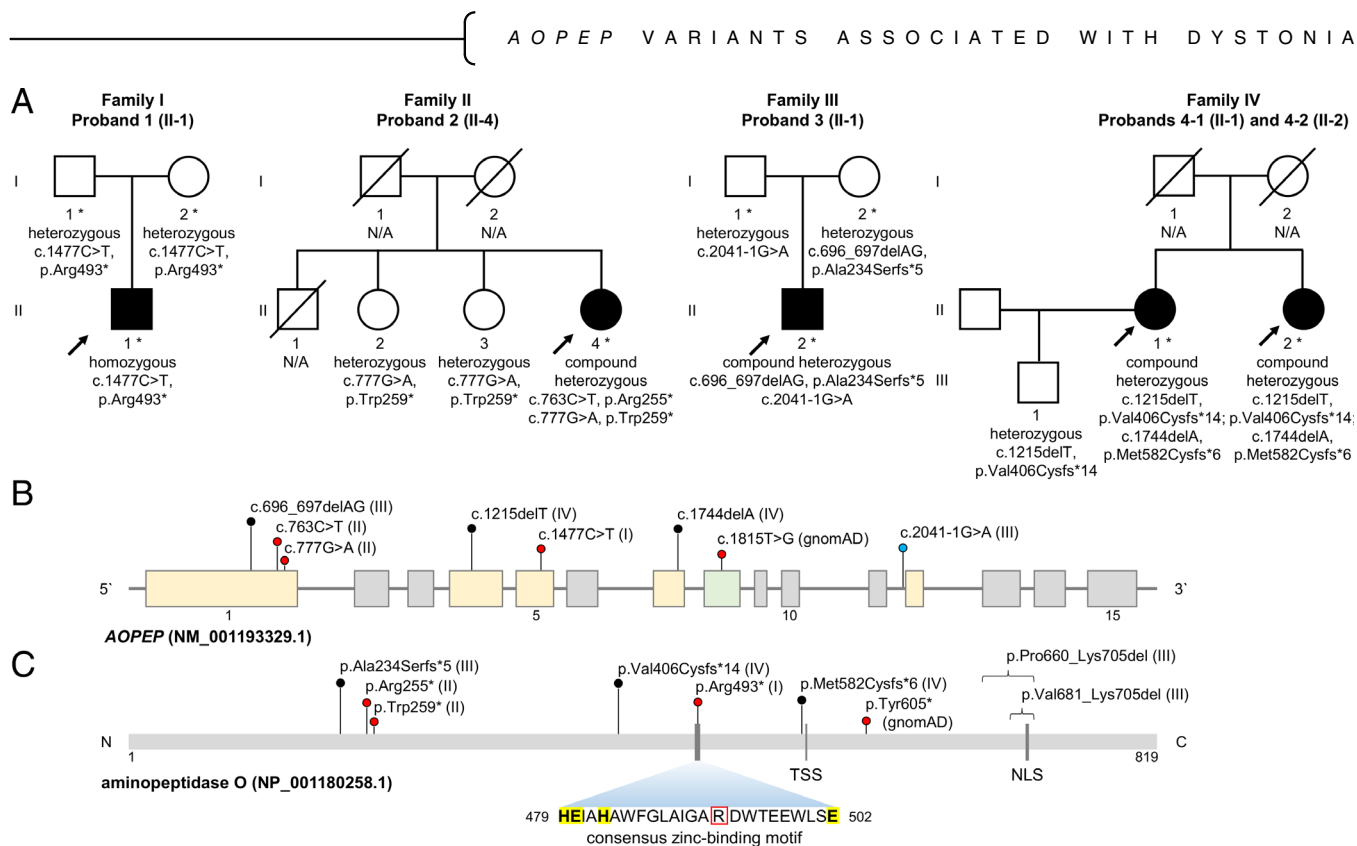
individuals can express dystonia without relevant comorbidity from childhood to late adulthood (sixth decade of life), uncovering a previously unrecognized recessively inherited isolated-dystonia syndrome. In one family, secondary late-onset parkinsonism was observed.

## Subjects and Methods

### Case Identification

The study started with the detection of a homozygous candidate variant in a participant (proband 1, family I; Fig. 1A) of a whole-exome sequencing study of unexplained dystonias.<sup>5,7</sup> Inclusion criteria, recruitment, and phenotyping strategies for this study have been reported in detail elsewhere.<sup>5,7</sup> In short, proband 1 was part of a research cohort recruited from specialized centers in seven European countries and assessed genetically at Helmholtz Center Munich and Technical University of Munich (Munich, Germany). The cohort comprised 953 index cases (54% female), of whom 55% had been diagnosed with isolated dystonia (without additional symptoms).

To identify additional individuals with variants in the gene of interest and a clinical phenotype similar to that



**FIG. 1.** Identification of predicted inactivating biallelic variants in *AOEPE* in four unrelated families with dystonia. **(A)** Pedigree drawings for families I–IV. Circles are female individuals, squares are male individuals, and slashes indicate deceased individuals; solid symbols represent subjects with dystonia (reported probands marked by an arrow), and open symbols are healthy family members. *AOEPE* genotypes are shown for all subjects for whom DNA was obtainable. Individuals who underwent genomic (exome or genome) sequencing are highlighted with asterisks. **(B)** Structural organization of human *AOEPE* (RefSeq: NM\_001193329.1; ENST00000375315.2) with the relative positions of each cDNA variant. Nonsense variants are indicated with red lollipops, while frameshift and splicing variants are indicated with black and blue lollipops, respectively. In addition to the proband-specific homozygous and compound heterozygous variants, a rare homozygous nonsense variant found in gnomAD (v2.1.1) is also illustrated. **(C)** Human aminopeptidase O schematic (Q8N6M6-1) with protein-level annotation of the variants shown in **(B)**. A magnified view of the HEXXH(X)<sub>18</sub>E zinc-binding motif is depicted, with the arginine residue affected by a homozygous nonsense variant in proband 1 highlighted with a red box. N/A, no DNA sample available; NLS, nucleolar localization signal; TSS, transition-state stabilizer. [Color figure can be viewed at [wileyonlinelibrary.com](http://onlinelibrary.wiley.com)]

of proband 1, we searched online matchmaking platforms (entry “*AOEPE*”)<sup>8</sup> and contacted independent investigators studying the genetic causality of dystonia. Genomic sequencing databases of the following collaborators’ institutions were interrogated: Phoenix Children’s Hospital (Phoenix, AZ, USA) and Yale Center for Genome Analysis (New Haven, CT, USA), Penn Neurogenetics Center (Philadelphia, PA, USA), UCL Great Ormond Street Institute of Child Health (London, UK), the Garvan Institute of Medical Research (Sydney, Australia), and the Ken and Ruth Davee Department of Neurology (Chicago, IL, USA).

Proband 2 (family II; Fig. 1A) was identified from a whole-genome sequencing project focused on exploring the causes of dystonic disorders (Sydney, New South Wales, Australia).<sup>4</sup> Details of the investigated cohort, consisting of 111 mostly isolated dystonia-affected individuals, have been described earlier.<sup>4</sup> Identification of proband 3 (family III; Fig. 1A) and probands 4-1/4-2 (family IV; Fig. 1A) was achieved through GeneMatcher.<sup>8</sup> Proband 3 was studied by whole-exome

sequencing in a program to find new phenotype–gene relationships at the University of Münster (Münster, Germany); the probands from family IV underwent whole-genome sequencing in the frame of the France Médecine Génomique 2025 plan (Paris, France).

All participating families I–IV had provided written informed consent under protocols approved by local research ethics committees.

## Genomic Explorations

All probands were exome or genome sequenced (trio-based whole-exome sequencing in families I and III; singleton whole-genome sequencing in family II; duo whole-genome sequencing in family IV). Target-enrichment, high-throughput sequencing and variant screenings were performed using established procedures<sup>4,5,7,9–12</sup> (for details, see Supporting Information Methods). In brief, libraries were generated using SureSelect Human All Exon V6 (families I and III), KAPA HyperPrep (family II), or NEB-Next Ultra II (family IV) kits, and sequenced on Illumina

platforms. Following standard bioinformatics annotations, variants were filtered by validated prioritization criteria, including protein-level effect, minor allele frequency, predicted deleteriousness, and suspected inheritance models. Causative variants in known disease genes were ruled out.<sup>4,5,9,11,12</sup>

### Variant Confirmation and cDNA Analysis

Using DNA samples from all available family members, candidate variants in *AOPEP* were confirmed by Sanger sequencing. Total RNA extracted from proband 3's whole blood (Qiagen) was reverse transcribed (Invitrogen) and amplified using primers targeting the cDNA region of interest. Products were fractionated with gel electrophoresis and Sanger sequenced.

## Results

### Clinical Findings

#### Proband 1 (Family I)

This 13-year-old boy was born full term to healthy parents of Italian ancestry after an uneventful pregnancy and delivery (Fig. 1A; Table 1). The neonatal period was unremarkable, and developmental milestones were reached normally. There was no relevant family history, and the parents reported no consanguinity. By the age of 9 years, the parents first noted that the boy's gait was impaired with unintentional inward rotation of his right leg. He was not able to suppress these movements and started complaining of fatigue while walking. He went on to experience stiffness of the first four fingers of his right hand and developed difficulty in handling little objects. Over the course of 6 months, symptoms progressed insidiously, and he became unable to write legibly without painful muscle spasms. After learning to perform manual tasks with his nondominant hand, he manifested left-finger cramps. Although involuntary movements spread to involve all four extremities, he remained independent in most daily activities. Diagnostic explorations, including routine laboratory testing of blood and cerebrospinal fluid samples, as well as neuroanatomic assessment with magnetic resonance imaging (MRI), were all unrevealing. Additional metabolic workup showed normal levels of copper, ceruloplasmin, lactate,  $\alpha$ -fetoprotein, vitamin E, thyroid hormones, urine organic acids, and plasma amino acids. Therapeutic trials with levodopa and anticholinergics yielded no benefit. The proband first presented to us (Medical University of Innsbruck, Innsbruck, Austria) at the age of 13 years. On neurological examination, we saw dystonic movements of both arms and forearms, worse with action and posture. There was dystonic wrist flexion and abnormal posturing of digits II–IV more pronounced on the right side when writing. His gait showed

proximal dystonia of the right leg and twisted foot postures bilaterally with slight instability. We also observed a tendency of shoulder abduction and truncal rotation while walking.

#### Proband 2 (Family II)

This 61-year-old European descent woman had no medical or family history of relevance (Fig. 1A; Table 1). Born to healthy nonconsanguineous parents, she manifested reduced dexterity, twisting of the neck and head, and gait instability since childhood. Her two healthy sisters were aged 73 and 75 years, respectively. She had normal motor development until 12 years of age, when she began to experience sustained contractions in her lower-limb musculature. Over the course of 3 years, symptoms progressed, affecting the right arm with fine motor skill impairment and forcing her to switch certain manual tasks to the left hand. She also experienced problems with handwriting, neck pain, and head deviation. After this initial period of deterioration, symptoms remained stable over the following decade. In subsequent years, mobility became increasingly difficult because of involuntary neck extension and abnormal foot posturing. Although she continued to work, she required the use of a wheelchair. In addition, slurred speech was progressively apparent. The proband was first seen by the adult neurology service at the age of 45 years and since has been assessed during regular follow-up visits. An array of unrevealing diagnostic evaluations was performed, including brain MRI, routine blood tests, electrophysiological studies, and targeted testing for *DYT-TOR1A*. Cerebrospinal fluid analysis revealed low levels of 5-hydroxyindoleacetic acid and bipterin, but normal neopterin concentrations. Treatment with anticholinergics and tetrabenazine alleviated symptoms to a small extent, whereas botulinum toxin injections (neck) had no effect. On her most recent examination (61 years old), proband 2 had marked retrocollis, intermittent right-sided torticollis, and finger/hand-flexion dystonia when arms were outstretched (Supporting Information Video 1). There was speech impairment presenting with dysarthrophonia and orofacial dyskinesia. Independent walking was compromised because of neck dystonia, but no overt lower-limb dystonic movements were observed.

#### Proband 3 (Family III)

This 58-year-old man, born from unrelated healthy parents of German origin after an uneventful pregnancy and delivery, had normal psychomotor development and was well during childhood and early adulthood (Fig. 1A; Table 1). There was no family history for movement disorders. Symptoms began at the age of 36 years with right hand action-dependent muscle cramps that soon progressed to involve the contralateral extremity. He reported significant writing

**TABLE 1** Clinical features of the five individuals with biallelic AOPEP variants

	Family I		Family II		Family III		Family IV	
	Proband 1	Proband 2	Proband 3	Proband 4-1	Proband 4-2	Proband 4-1	Proband 4-2	
AOPEP variant(s) (NM_NLM_001193329.1, NP_001180258.1)	c.1477C>T (p.Arg493*); c.763C>T (p.Arg255*); c.777G>A (p.Trp259*)	c.763C>T (p.Arg255*); c.777G>A (p.Trp259*)	c.696_697delAG (p.Ala234Serfs*5); c.2041-1G>A	c.1215delT (p.Val406Cysfs*14); c.1744delA (p.Met582Cysfs*6)	c.1215delT (p.Val406Cysfs*14); c.1744delA (p.Met582Cysfs*6)	c.1215delT (p.Val406Cysfs*14); c.1744delA (p.Met582Cysfs*6)	c.1215delT (p.Val406Cysfs*14); c.1744delA (p.Met582Cysfs*6)	
Genomic testing strategy	Parent-proband trio whole-exome sequencing	Proband-only whole-genome sequencing	Parent-proband trio whole-exome sequencing	Duo whole-genome sequencing	Duo whole-genome sequencing	Duo whole-genome sequencing	Duo whole-genome sequencing	
Age, y	13	61	58	61	52	61	52	
Sex	Male	Female	Male	Female	Female	Female	Female	
Geographic origin (ethnicity)	Italy (European)	Australia (European)	Germany (European)	France (European)	France (European)	France (European)	France (European)	
Family history	Negative	Negative	Negative	Positive (two affected sisters)	Positive (two affected sisters)	Positive (two affected sisters)	Positive (two affected sisters)	
Consanguinity	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	
<b>Dystonia characteristics</b>								
Age at onset	Childhood (9 y)	Childhood (12 y)	Adulthood (36 y)	Adulthood (22 y)	Adulthood (24 y)	Adulthood (22 y)	Adulthood (24 y)	
Site of onset	Lower limb	Lower limb	Upper limb	Neck	Upper limb	Neck	Upper limb	
Disease course	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive	
Symptom variability	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	Persistent and action specific (writer's cramp)	
Body distribution (most recent examination)	Generalized	Multifocal	Multifocal	Generalized	Generalized	Generalized	Generalized	
Involved areas (most recent examination)	Upper limbs, lower limbs, trunk	Face, neck, upper limbs	Face, upper limbs, lower limbs	Face, upper limbs, lower limbs, trunk	Neck, upper limbs, lower limbs, trunk	Neck, upper limbs, lower limbs, trunk	Neck, upper limbs, lower limbs, trunk	
Tremor	No	No	No	No	No	No	No	
Dystonia syndrome	Early-onset generalized isolated dystonia	Early-onset multifocal isolated dystonia	Multifocal isolated dystonia with onset in adulthood	Generalized combined dystonia with onset in adulthood	Generalized combined dystonia with onset in adulthood	Generalized combined dystonia with onset in adulthood	Generalized combined dystonia with onset in adulthood	
<b>Associated features</b>								
Other movement disorders	No	No	No	No	No	No	No	
				Parkinsonism (akinesia, freezing of gait/falls, dysarthria)	Parkinsonism (akinesia, freezing of gait/falls, dysarthria)	Parkinsonism (akinesia, freezing of gait/falls, dysarthria)	Parkinsonism (akinesia, freezing of gait/falls, dysarthria)	

(Continues)

TABLE 1 Continued

	Family I Proband 1		Family II Proband 2		Family III Proband 3		Family IV Proband 4-1		Proband 4-2		
	Other neurological/ psychiatric features	No	No	No	No	No	No	No	No	Depression	
Systemic features	No	No	No	No	No	No	No	No	No		
<b>Additional clinical information</b>											
Brain magnetic resonance imaging abnormality	No	No	No	No	No	No	No	No	No		
Tried medications, therapy	Levodopa (no response), anticholinergics (no response)	Levodopa (no response), anticholinergics (partial response), tetabenazine (partial response), botulinumtoxin (no response)	Anticholinergics (partial response), tetabenazine (partial response), botulinumtoxin (no response)	Anticholinergics (no response), botulinumtoxin (no response)	Levodopa (no response), anticholinergics (no response), botulinumtoxin (no response)	Levodopa (no response), anticholinergics (no response), botulinumtoxin (no response)	Levodopa (no response), botulinumtoxin (no response), baclofen (partial response)	Pallidal deep brain stimulation (partial response), levodopa (partial response on gait impairment)	Levodopa (no response), botulinumtoxin (no response), baclofen (partial response)		

difficulties and over the course of a few years, overlapping fingers (digits III–V on the left hand) and persistently extended digit II (on the left) led to disturbances in selective grasping. By age 46 years, he had developed distal lower-limb abnormal posturing, causing occasional gait imbalance, followed by the appearance of uncontrollable facial movements and masseter muscle hypertrophy. The proband showed impairments in swallowing function resulting in recurrent aspirations. In recent years, he also noticed increasing articulation deficits. Speech problems improved to a certain extent with a sensory trick, whereas his condition was aggravated by fatigue and stress. Thorough diagnostic workup was normal, including brain MRI, positron emission tomography/computed tomography, and blood laboratory tests. Several medications were administered without sustained benefit: anticholinergics, levodopa, and botulinum toxin injections. Neurological examination at the age of 57 years (University of Münster, Münster, Germany) revealed dystonic postures in his fingers associated with overflow muscle activation, both-sided writer's cramp, facial dyskinesia predominantly affecting the lower hemiface, and marked dysarthrophonia. His gait was interrupted by sudden bilateral muscle spasms, but no continuous dystonic movements in his legs were documented.

#### Probands 4-1 and 4-2 (Family IV)

The affected sisters were born to healthy unrelated parents of French descent (Fig. 1A; Table 1). Proband 4-1 is a 61-year-old woman without relevant medical history during childhood and adolescence. She developed left laterocollis at age 22 years, writing difficulties at age 34, and by the age of 36 she had generalized dystonia with four-limb dystonia and trunk involvement. She was treated with deep brain stimulation at the age of 41 years, with improvement of limb dystonia severity and disability, but only partial improvement of trunk dystonia. At the age of 58 years, she experienced development of parkinsonism (mainly akinesia) and worsening of dysarthria. She manifested progressive walking difficulties with freezing of gait and had to use a walking aid. Increasing gait abnormalities were initially thought to be related to long-term effects of neurostimulation<sup>13</sup>; dopaminergic denervation was subsequently discovered on DaTscan, and she was treated with levodopa (600 mg/day) with partial gait improvement. At last examination (61 years old; Hôpital Pitié-Salpêtrière, Paris, France), she presented generalized dystonia with signs of parkinsonism, including right-sided akinesia, right-foot freezing and festination, along with dysarthria and hypophonia (Supporting Information Video 2). Proband 4-2, her 52-year-old sister, had no medical problems during childhood and early adulthood. Her first symptoms occurred at the age of

**TABLE 2** Genetic description of the AOPEP variants identified in families I–IV

Family	Genomic variant <sup>a</sup>	Exon <sup>b</sup>	cDNA variant <sup>b</sup>	Protein variant <sup>b</sup>	Variant class	Variant type	Zygoty	dbSNP	Frequency gnomAD (allele count/total allele number)	Frequency in-house control exomes (allele count/total allele number)	LOFTEE
I	chr9:9768380C>T	5	c.1477C>T	p.Arg493*	Loss of function	Nonsense	Homozygous	rs577000059	2/154,298; no homozygotes detected	Not found	HC
II	chr9:97522828C>T	1	c.763C>T	p.Arg255*	Loss of function	Nonsense	Compound heterozygous	rs779393035	6/200,132; no homozygotes detected	1/40,000; no homozygotes detected	HC
III	chr9:97522761-97522762delAG	1	c.777G>A	p.Trp259*	Loss of function	Nonsense	Compound heterozygous	rs1043405296	Not found	Not found	HC
	chr9:97767823G>A	12	c.2041-1G>A	p.Val681_Lys705del; p.Pro660_Lys705del	Loss of function	Splice-site	Compound heterozygous	rs141221108	19/282,436; no homozygotes detected	2/40,000; no homozygotes detected	HC
IV	chr9:97563135delTT	4	c.1215delT	p.Val406Cysfs*14	Loss of function	Frameshift	Compound heterozygous	rs747916174	14/251,418; no homozygotes detected	1/40,000; no homozygotes detected	HC
	chr9:97717541delA	7	c.1744delA	p.Met582Cysfs*6	Loss of function	Frameshift	Compound heterozygous	rs778493729	6/281,778; no homozygotes detected	1/40,000; no homozygotes detected	HC

gnomAD, Genome Aggregation Database (v2.1.1); LOFTEE, Loss-of-Function Transcript Effect Estimator; HC, high confidence.

<sup>a</sup>The reference genome used is GRCh37/hg19.

<sup>b</sup>Numbering according to National Center for Biotechnology Information accession numbers NM\_001193329.1 and NP\_001180258.1.

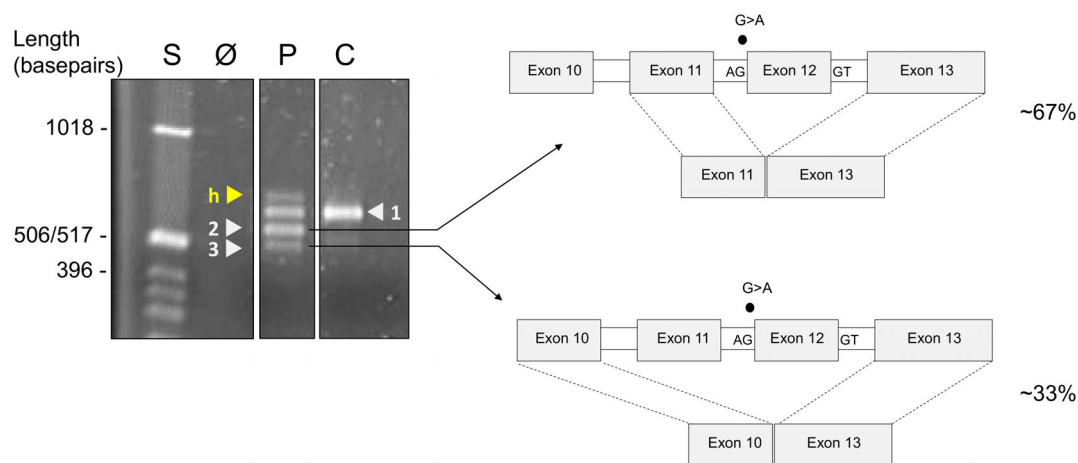
24 years, when she reported writing difficulties with excessive pressure of the thumb and index fingers. She progressively developed dystonic dysarthria, which gradually became invalidating. Task-specific dystonia evolved toward the impairment of other activities and bilateral upper-limb dystonia. At the age of 47 years, she experienced gait difficulties with recurrent backward falls, and cervical/truncal dystonia at the age of 51 years. She became akinetic. DaTscan performed at age 52 years did not show overt dopaminergic loss. Brain and spinal MRI were normal. She had no beneficial effect of levodopa and botulinum toxin, but mild response to baclofen. Examination at the age of 52 years demonstrated generalized dystonia combined with an akinetic syndrome; she had dysarthria with moderate hypophonia.

### Genomic Findings

Through whole-exome and whole-genome sequencing, the probands were identified to carry four different biallelic variants, totaling seven different nonsense, frameshift, and splice-site variants in *AOPEP*, a gene with largely unexplored function and no previous link to any Mendelian phenotype (Fig. 1A–C; Table 2). All variants were expected to be the target of nonsense-mediated decay or, in case of escape, to cause truncated protein products lacking essential functional sites. *In silico* evaluation using the Loss-of-Function Transcript Effect Estimator<sup>14</sup> indicated that all variants were part of the high-confidence set of predicted loss-of-function alleles in the genome (Table 2). None of the probands had other rare variants fulfilling criteria for a “pathogenic” or “likely pathogenic” allele<sup>15</sup> in any previously described genes

linked to dystonia. Variant nomenclature provided in the following refers to RefSeq accession numbers NM\_001193329.1 and NP\_001180258.1.

Proband 1 had a homozygous c.1477C>T substitution in exon 5, expected to lead to premature stop of translation at amino acid position 493 (p.Arg493\*). By screening the proband’s exome data for runs of homozygosity, we detected one contiguous homozygous stretch of ~11.8 Mb on chromosome 9 (chr9:93,606,309–105,405,414) containing c.1477C>T. The variant was reported twice in Genome Aggregation Database (gnomAD v2.1.1),<sup>14</sup> but not in a homozygous state. The parents were each obligate carriers of the variant. In proband 2, we uncovered the substitutions c.763C>T and c.777G>A, both of which were predicted to result in the insertion of a premature termination codon within exon 1 (p.Arg255\* and p.Trp259\*, respectively). The c.763C>T variant was listed six times in gnomAD-v2.1.1 (zero homozygotes),<sup>14</sup> whereas c.777G>A was absent from this database. A compound heterozygous status of the variants was confirmed by visual inspection of read alignments from the proband’s sequencing data (Supporting Information Fig. 1). Two healthy siblings of proband 2 were found to harbor the c.777G>A variant, but not the c.763C>T allele (Fig. 1A). Proband 3 was found to possess a deletion of two nucleotides (c.696\_697delAG) in exon 1, creating a frameshift and the introduction of a premature stop codon five triplets downstream (p.Ala234Serfs\*5), in combination with a substitution in the acceptor splice site of intron 11 (c.2041-1G>A). The frameshift variant was seen in nine carriers from gnomAD-v2.1.1 and the splice-site variant in 19 carriers, while neither variant was present in a homozygous state in this database.<sup>14</sup> The c.696\_697delAG variant was transmitted from the proband’s mother and



**FIG. 2.** Proband 3’s c.2041-1G>A allele in *AOPEP* produces a splicing defect. cDNA samples of the proband (P) and a control subject (C) were polymerase chain reaction (PCR) amplified using primers located in exons 7 and 14, respectively. The control sample showed the expected signal at 678 bp (label 1). Sequencing demonstrated that signal 2 is due to skipping of exon 12, while signal 3 is due to skipping of exons 11 and 12. Signal “h” (yellow) is most likely a heteroduplex of, for example, products 1 and 2 with correspondingly reduced mobility. S: length standard. Boxes represent cropped sections from a digital image of a single gel: two additional lanes of the original gel representing the same PCR using 5-fold more template and demonstrating consequently a more intense heteroduplex signal are not shown. The exon-skipping events are illustrated with light gray boxes (right panel). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



c.2041-1G>A from his father, consistent with autosomal recessive inheritance. Analysis of cDNA demonstrated that the c.2041-1G>A variant resulted in a missplicing event, producing two different aberrant products: one lacking exon 12 (~2/3 of the signal) and another lacking exons 11 and 12 (~1/3 of the signal) (Fig. 2). Skipping of exon 12 and of exons 11 plus 12 were predicted to generate products with larger in-frame deletions: p.Val681\_Lys705del (exon 12 deletion) and p.Pro660\_Lys705del (deletion of exons 11 and 12). Proband 4-1 and 4-2 were identified with two frameshift variants: c.1215delT in exon 4 and c.1744delA in exon 7 (p.Val406Cysfs\*14 and p.Met582Cysfs\*6, respectively). Both variants were present in gnomAD-v2.1.1 (c.1215delT in 14 carriers and c.1744delA in 6 carriers), but not in homozygosity. Analysis of DNA from proband 4-1's healthy son revealed the presence of only the c.1215delT allele, establishing a compound heterozygous status of the variants in the probands (Fig. 1A).

## Discussion

Because most monogenic neurological disorders are individually rare, the study of very large patient cohorts is often warranted to connect independent individuals with matching genotypic profiles.<sup>6,16,17</sup> We combined genomic sequencing data from 11 countries on three continents to identify *AOPEP* biallelic loss-of-function variants in four unrelated pedigrees with dystonia. Our approach, beginning with the discovery of a promising candidate variant in a single subject from a whole-exome sequencing-based research cohort,<sup>5,7</sup> reinforces the need for extended collaboration between centers and sharing of genomic findings to enhance knowledge about rare genetically mediated dystonic conditions.<sup>16</sup>

*AOPEP* encodes aminopeptidase O (AP-O), an intracellular member of the M1 family of metalloproteases.<sup>18,19</sup> M1 aminopeptidases constitute a conserved set of multifunctional enzymes, defined by the presence of a consensus zinc-binding motif (HEXXH[X]<sub>18</sub>E).<sup>20</sup> Within the active site, two histidines (H) and a distal glutamate (E) coordinate the interaction between the enzyme and its essential cofactor zinc.<sup>20</sup> Catalyzing the removal of N-terminal amino acids from various substrate targets in a zinc-dependent manner, M1 aminopeptidases govern diverse processes, including protein turnover, cell-cycle control, reproduction and development, neuropeptide generation, signaling, and defense.<sup>18</sup> Although distributed in a wide range of tissue types, M1 aminopeptidases are subject to strict spatial and temporal regulation.<sup>18,21</sup> AP-O is ubiquitously expressed in adult human brain tissue,<sup>22,23</sup> including the cerebral cortex, basal ganglia, and cerebellum, as well as during human brain development.<sup>24</sup> Several alternative isoforms of the protein exist, with transcript

variant 1 (canonical transcript NM\_001193329.1) coding for the longest (isoform-1; 819 amino acid protein; UniProt: Q8N6M6-1).<sup>25</sup> In addition to the HEXXH(X)<sub>18</sub>E zinc-binding motif (amino acids 479–502), AP-O isoform-1 is organized into a transition-state stabilizer site (amino acid 586), critically required for enzymatic catalysis, and a nucleolar localization signal (amino acids 689–699).<sup>19,25</sup> Information about the physiological roles of AP-O remains fragmentary. One study reported that the enzyme is involved in vascular cell biology,<sup>19</sup> but the relevance of AP-O with regard to brain function has not been elucidated to date. Although mice deficient for AP-O were described as being neurologically normal,<sup>26</sup> it is interesting to consider that bioactive peptides produced by proteolytic processing enzymes of the M1 family exert profound effects on neural cell development and viability.<sup>21,27</sup> For example, leucyl-cystinyl aminopeptidase, another brain-expressed M1 aminopeptidase, is known to participate in a broad range of aspects related to synaptogenesis, long-term potentiation, and neuroprotection.<sup>28</sup> Although currently speculative, it is conceivable that defects of such processes could contribute to the disease expressions of our probands with *AOPEP* variants.<sup>29</sup> A recent study based on transcriptomic data from adult brain tissue highlighted the existence of gene networks expressed in the striatum, substantia nigra, and frontal cortex that are significantly enriched for both genes involved in synaptic signaling and several dystonia-related genes, hence suggesting functional convergence of these dystonia-causing genes in regulating this essential neuronal function.<sup>30</sup> Importantly, although *AOPEP* is not part of these gene networks, this does not exclude its role in dystonia pathogenesis, because several other established dystonia-related genes were not found to be part of these networks (ie, *TOR1A*, *THAP1*, and *KMT2B*).<sup>30</sup> This may reflect the fact that these genes are more likely to play a critical role for dystonia pathogenesis during brain development and less so in the adult mature brain.<sup>30</sup> Single-nucleotide polymorphism-based association studies have shown that more common variations in *AOPEP* may influence susceptibility to prevalent disease traits, such as arterial hypertension, polycystic ovary syndrome, and atrial fibrillation.<sup>31–33</sup> We believe, however, that it is currently not possible to bring these observations into a direct pathophysiological context with dystonia because the herein identified loss-of-function alterations are likely to have molecular consequences different from those encountered in association to common (non-neurological) disease risk alleles.

Two key arguments support the contention that the *AOPEP* variants identified in this study are causally linked to the probands' phenotypes. First, no putative pathogenic variants in known disease genes or any additional candidate gene were observed; the variants were rare, not found in a homozygous status in controls, and segregated in the probands as expected for an

autosomal recessive trait. All variants were predicted to cause a loss of function via nonsense-mediated decay and/or disruption of functionally important sites of AP-O; in particular, the nonsense variants (proband 1 and 2) and the frameshift variants (proband 3 and 4-1/4-2) were expected to deleteriously affect the zinc-binding motif and/or the transition-state stabilizer, both essential for enzymatic activity (Fig. 1C). Protein products resulting from the splice-variant allele (proband 3) were expected to exhibit selective loss of the nucleolar localization signal (Fig. 1C). Second, our genotype-first approach allowed us to connect four unrelated families with strongly overlapping phenotypes. All probands shared a strikingly similar presentation of dystonia that progressed within 3–14 years to involve both their upper and lower extremities. Dystonic symptoms were described as severely disabling, causing substantial impairments in daily living. In four probands, dystonia also involved the craniocervical districts, with abnormal orofacial movements, cervical dystonia, and dysarthric speech. Onset occurred in childhood in two probands, whereas probands 3 and 4-1/4-2 manifested first symptoms in adulthood. The relatively late onset of dystonic features in proband 3 could be attributable to the nature of his c.2041-1G>A allele, possibly retaining some enzymatic activity. Notably, probands 1–3 had no evidence of coexisting neurological or systemic involvement and were diagnosed with isolated dystonia. In contrast, the probands from the fourth family had prominent dystonic disorders with secondary evolution of late-onset parkinsonism.

We were unable to identify any further biallelic loss-of-function variants in *AOPEP* in 20,000 in-house exomes of individuals with unrelated clinical conditions (Munich, Germany),<sup>34</sup> as well as in other genomic databases examined as part of this work (see Methods). However, one carrier of an *AOPEP* homozygous nonsense c.1815T>G (p.Tyr605\*) substitution was found in the Pakistani PROMIS cohort<sup>35</sup> and is listed in gnomAD (v2.1.1).<sup>14</sup> This observation does not argue against a disease-causing nature of the *AOPEP* variants detected in our probands for several reasons. First, the gnomAD-annotated variant is expected to fall into a more C-terminally located portion of AP-O than most of the proband-specific variants, leaving the zinc-binding and the transition-state stabilizer sites intact (Fig. 1C). Second, it is possible that alternative splicing of variant-bearing *AOPEP* transcripts gives rise to different molecular and phenotypic outcomes. Unlike the proband-specific variants identified in this study, the gnomAD-annotated variant does not affect certain AP-O protein products, such as isoform-3 (UniProt: Q8N6M6-3).<sup>25</sup> Third, because of the recruitment strategy in the PROMIS study focusing on myocardial infarction phenotypes, it is also possible that this cohort contains individuals with additional noncardiac symptoms, for example,

individuals with adult-onset movement disorders.<sup>35</sup> Two *AOPEP* biallelic frameshift variants are also reported in gnomAD (v2.1.1 and/or v3.1.1), but these alterations fall into the introns of the canonical isoform NM\_001193329.1 and affect only alternative short isoforms (low-confidence, flagged variant alleles according to Loss-of-Function Transcript Effect Estimator<sup>14</sup>), which is in strong favor of benignity.<sup>15</sup> Analyses of neural cell models would be required to determine the precise molecular consequences of different *AOPEP* loss-of-function variants and to assess the specific patterns of AP-O isoform expression.

In summary, we describe five individuals manifesting a similar presentation of progressive dystonia with prominent limb involvement and propose that their phenotypes are attributable to recessively inherited loss-of-function variants in *AOPEP*. Identification of further cases with dystonia and *AOPEP* variants will be integral to confirm our findings and provide more insight into the spectrum of clinical phenotypes associated with loss of human AP-O function. ■

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## Data Availability Statement

Data available on request from the authors.

## References

- Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord* 2013;28(7): 863–873.
- Balint B, Mencacci NE, Valente EM, et al. Dystonia. *Nat Rev Dis Primers* 2018;4(1):25.
- Keller Sarmiento IJ, Mencacci NE. Genetic dystonias: update on classification and new genetic discoveries. *Curr Neurol Neurosci Rep* 2021;21(3):8.
- Kumar KR, Davis RL, Tchan MC, et al. Whole genome sequencing for the genetic diagnosis of heterogenous dystonia phenotypes. *Parkinsonism Relat Disord* 2019;69:111–118.

5. Zech M, Jech R, Boesch S, et al. Monogenic variants in dystonia: an exome-wide sequencing study. *Lancet Neurol* 2020;19(11):908–918.
6. Kaplanis J, Samocha KE, Wiel L, et al. Evidence for 28 genetic disorders discovered by combining healthcare and research data. *Nature* 2020;586(7831):757–762.
7. Zech M, Boesch S, Skorvanek M, et al. Clinically relevant copy-number variants in exome sequencing data of patients with dystonia. *Parkinsonism Relat Disord* 2021;84:129–134.
8. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat* 2015;36(10):928–930.
9. Zech M, Boesch S, Jochim A, et al. Clinical exome sequencing in early-onset generalized dystonia and large-scale resequencing follow-up. *Mov Disord* 2017;32(4):549–559.
10. Kumar KR, Wali GM, Kamate M, et al. Defining the genetic basis of early onset hereditary spastic paraplegia using whole genome sequencing. *Neurogenetics* 2016;17(4):265–270.
11. Park JH, Elpers C, Reunert J, et al. SOD1 deficiency: a novel syndrome distinct from amyotrophic lateral sclerosis. *Brain* 2019;142(8):2230–2237.
12. de Sainte Agathe JM, Mercier S, Mahe JY, et al. RNF170-related hereditary spastic paraplegia: confirmation by a novel mutation. *Mov Disord* 2021;36(3):771–774.
13. Kosutzka Z, Rivaud-Pechoux S, Pouget P, et al. Pathophysiology of gait disorders induced by bilateral globus pallidus interna stimulation in dystonia. *Brain* 2020;143(1):e3.
14. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581(7809):434–443.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405–424.
16. Steel D, Zech M, Zhao C, et al. Loss-of-function variants in HOPS complex genes VPS16 and VPS41 cause early onset dystonia associated with lysosomal abnormalities. *Ann Neurol* 2020;88(5):867–877.
17. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. *Nature* 2015;519(7542):223–228.
18. Drinkwater N, Lee J, Yang W, Malcolm TR, McGowan S. M1 aminopeptidases as drug targets: broad applications or therapeutic niche? *FEBS J* 2017;284(10):1473–1488.
19. Axton R, Wallis JA, Taylor H, Hanks M, Forrester LM. Aminopeptidase O contains a functional nucleolar localization signal and is implicated in vascular biology. *J Cell Biochem* 2008;103(4):1171–1182.
20. Albiston AL, Ye S, Chai SY. Membrane bound members of the M1 family: more than aminopeptidases. *Protein Pept Lett* 2004;11(5):491–500.
21. Hui KS. Brain-specific aminopeptidase: from enkephalinase to protector against neurodegeneration. *Neurochem Res* 2007;32(12):2062–2071.
22. Thul PJ, Lindskog C. The human protein atlas: a spatial map of the human proteome. *Protein Sci* 2018;27(1):233–244.
23. Gamazon ER, Segre AV, van de Bunt M, et al. Using an atlas of gene regulation across 44 human tissues to inform complex disease- and trait-associated variation. *Nat Genet* 2018;50(7):956–967.
24. Miller JA, Ding SL, Sunkin SM, et al. Transcriptional landscape of the prenatal human brain. *Nature* 2014;508(7495):199–206.
25. The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Res* 2017;45(D1):D158–D169.
26. Eppig JT. Mouse genome informatics (MGI) resource: genetic, genomic, and biological knowledgebase for the laboratory mouse. *ILAR J* 2017;58(1):17–41.
27. Kudo LC, Parfenova L, Ren G, et al. Puromycin-sensitive aminopeptidase (PSA/NPEPPS) impedes development of neuropathology in hPSA/TAU(P301L) double-transgenic mice. *Hum Mol Genet* 2011;20(9):1820–1833.
28. Paladini F, Fiorillo MT, Tedeschi V, Matorre B, Sorrentino R. The multifaceted nature of aminopeptidases ERAP1, ERAP2, and LNPEP: from evolution to disease. *Front Immunol* 2020;11:1576.
29. Gonzalez-Latapi P, Marotta N, Mencacci NE. Emerging and converging molecular mechanisms in dystonia. *J Neural Transm (Vienna)* 2021;128(4):483–498.
30. Mencacci NE, Reynolds R, Ruiz SG, et al. Dystonia genes functionally converge in specific neurons and share neurobiology with psychiatric disorders. *Brain* 2020;143(9):2771–2787.
31. Ichikawa M, Konoshita T, Makino Y, Suzuki J, Ishizuka T, Nakamura H. An association study of C9orf3, a novel component of the renin-angiotensin system, and hypertension in diabetes. *Sci Rep* 2020;10(1):16111.
32. Cui L, Li G, Zhong W, et al. Polycystic ovary syndrome susceptibility single nucleotide polymorphisms in women with a single PCOS clinical feature. *Hum Reprod* 2015;30(3):732–736.
33. Martin RI, Babaei MS, Choy MK, et al. Genetic variants associated with risk of atrial fibrillation regulate expression of PITX2, CAV1, MYOZ1, C9orf3 and FANCC. *J Mol Cell Cardiol* 2015;85:207–214.
34. Brunet T, Jech R, Brugger M, et al. De novo variants in neurodevelopmental disorders—experiences from a tertiary care center. *Clin Genet* 2021;100(1):14–28.
35. Saleheen D, Natarajan P, Armean IM, et al. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* 2017;544(7649):235–239.

## Supporting Data

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