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

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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.¹ 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.² With the widespread use of comprehensive genomic analysis techniques, progress has been made in the elucidation of monogenic causes of isolated dystonia.³ Nevertheless, 85%–95% of affected individuals remain without an etiologic diagnosis after the application of whole-exome or whole-genome sequencing.⁴,⁵ In recent years, Mendelian disease gene discovery has been substantially enhanced by international collaborative matchmaking efforts and pooling of cases with variant families. The study started with the detection of a homozygous candidate variant in a participant (proband 1, family 1; Fig. 1A) of a whole-exome sequencing study of unexplained dystonias.⁶,⁷ Inclusion criteria, recruitment, and phenotyping strategies for this study have been reported in detail elsewhere.⁶,⁷ 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 variants in the-gene of interest and a clinical phenotype similar to that...
of proband 1, we searched online matchmaking platforms (entry “AOPEP”) 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). Details of the investigated cohort, consisting of 111 mostly isolated dystonia-affected individuals, have been described earlier. Identification of proband 3 (family III; Fig. 1A) and probands 4-1/4-2 (family IV; Fig. 1A) was achieved through GeneMatcher. 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 Médicine 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 (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 NEBNext 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.4,5,9,11,12

**Variant Confirmation and cDNA Analysis**

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

(Continues)
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; 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>
<thead>
<tr>
<th>Family</th>
<th>Genomic variant(^a)</th>
<th>Exon(^b)</th>
<th>cDNA variant(^b)</th>
<th>Protein variant(^b)</th>
<th>Variant class</th>
<th>Variant type</th>
<th>Zygosity</th>
<th>dbSNP</th>
<th>Frequency gnomAD (allele count/total allele number)</th>
<th>Frequency in-house control exomes (allele count/total allele number)</th>
<th>LOFTEE</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>chr:97686380 C&gt;T</td>
<td>5</td>
<td>c.1477C&gt;T</td>
<td>p.Arg493*</td>
<td>Loss of function</td>
<td>Nonsense</td>
<td>Homozygous</td>
<td>rs577000059</td>
<td>2/154,298; no homozygotes detected</td>
<td>Not found</td>
<td>HC</td>
</tr>
<tr>
<td>II</td>
<td>chr:97522828 C&gt;T</td>
<td>1</td>
<td>c.763C&gt;T</td>
<td>p.Arg255*</td>
<td>Loss of function</td>
<td>Nonsense</td>
<td>Compound heterozygous</td>
<td>rs779393035</td>
<td>6/200,132; no homozygotes detected</td>
<td>1/40,000; no homozygotes detected</td>
<td>HC</td>
</tr>
<tr>
<td></td>
<td>chr:97522842 G&gt;A</td>
<td>1</td>
<td>c.777G&gt;A</td>
<td>p.Trp259*</td>
<td>Loss of function</td>
<td>Nonsense</td>
<td>Compound heterozygous</td>
<td>Not found</td>
<td>Not found</td>
<td>HC</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>chr:97522761-97522762 delAG</td>
<td>1</td>
<td>c.696_697delAG</td>
<td>p.Ala234Serfs*5</td>
<td>Loss of function</td>
<td>Frameshift</td>
<td>Compound heterozygous</td>
<td>rs750720840</td>
<td>9/250,236; no homozygotes detected</td>
<td>1/40,000; no homozygotes detected</td>
<td>HC</td>
</tr>
<tr>
<td></td>
<td>chr:97767823 G&gt;A</td>
<td>12</td>
<td>c.2041-1G&gt;A</td>
<td>p.Val681_Lys705del; p.Pro660_Lys705del</td>
<td>Loss of function</td>
<td>Splice-site</td>
<td>Compound heterozygous</td>
<td>rs141221108</td>
<td>19/282,436; no homozygotes detected</td>
<td>2/40,000; no homozygotes detected</td>
<td>HC</td>
</tr>
<tr>
<td>IV</td>
<td>chr:97563135 delT</td>
<td>4</td>
<td>c.1215delT</td>
<td>p.Val406Cysfs*14</td>
<td>Loss of function</td>
<td>Frameshift</td>
<td>Compound heterozygous</td>
<td>rs747916174</td>
<td>14/251,418; no homozygotes detected</td>
<td>1/40,000; no homozygotes detected</td>
<td>HC</td>
</tr>
<tr>
<td></td>
<td>chr:97717541 delA</td>
<td>7</td>
<td>c.1744delA</td>
<td>p.Met582Cysfs*6</td>
<td>Loss of function</td>
<td>Frameshift</td>
<td>Compound heterozygous</td>
<td>rs778493729</td>
<td>6/281,778; no homozygotes detected</td>
<td>1/40,000; no homozygotes detected</td>
<td>HC</td>
</tr>
</tbody>
</table>

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

\(\text{\(^a\)The reference genome used is GRCh37/hg19.}\)

\(\text{\(^b\)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 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 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 \( \sim 11.8 \text{Mb} \) 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), 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), 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. The c.696_697delAG variant was transmitted from the proband’s mother and

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**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]
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). Probands 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.\(^6,6,16,17\) 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,\(^5,7\) reinforces the need for extended collaboration between centers and sharing of genomic findings to enhance knowledge about rare genetically mediated dystonic conditions.\(^6\)

AOPEP encodes aminopeptidase O (AP-O), an intracellular member of the M1 family of metalloproteases.\(^18,19\) M1 aminopeptidases constitute a conserved set of multifunctional enzymes, defined by the presence of a consensus zinc-binding motif (HEXXH[X]\(_{18}\)E).\(^10\) Within the active site, two histidines (H) and a distal glutamate (E) coordinate the interaction between the enzyme and its essential cofactor zinc.\(^20\) 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.\(^18\) Although distributed in a wide range of tissue types, M1 aminopeptidases are subject to strict spatial and temporal regulation.\(^18,21\) AP-O is ubiquitously expressed in adult human brain tissue,\(^22,23\) including the cerebral cortex, basal ganglia, and cerebellum, as well as during human brain development.\(^24\) 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).\(^23\) In addition to the HEXXH[X]\(_{18}\)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).\(^19,25\) Information about the physiological roles of AP-O remains fragmentary. One study reported that the enzyme is involved in vascular cell biology,\(^19\) 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,\(^26\) 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.\(^21,27\) 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.\(^28\) Although currently speculative, it is conceivable that defects of such processes could contribute to the disease expressions of our probands with AOPEP variants.\(^29\) 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.\(^30\) 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).\(^30\) 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.\(^30\) 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.\(^31-33\) 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 (probands 1 and 2) and the frameshift variants (probands 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 cranio-cervical 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), 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 and is listed in gnomAD (v2.1.1). 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). This, 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. 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), which is in strong favor of benignity. 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


Supporting Data

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

M.Z.: study design and concept, acquisition of data, analysis and interpretation of data, and wrote manuscript.
K.R.K.: study design and concept, acquisition of data, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
S.R.: study design and concept, acquisition of data, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
J.R.: acquisition of data and revision of manuscript for critical intellectual content.
M.T.: acquisition of data and revision of manuscript for critical intellectual content.
L.G.R.: acquisition of data and revision of manuscript for critical intellectual content.
A.P.D.: acquisition of data and revision of manuscript for critical intellectual content.
R.J.A: acquisition of data and revision of manuscript for critical intellectual content.
R.B.: acquisition of data and revision of manuscript for critical intellectual content.
S. Biskup: acquisition of data and revision of manuscript for critical intellectual content.
N.D.: acquisition of data and revision of manuscript for critical intellectual content.
S. Bakhtiari: acquisition of data and revision of manuscript for critical intellectual content.
S.C.J.: acquisition of data and revision of manuscript for critical intellectual content.
M.C.K.: acquisition of data and revision of manuscript for critical intellectual content.
T.B.: acquisition of data and revision of manuscript for critical intellectual content.
P.G.-A.: acquisition of data and revision of manuscript for critical intellectual content.
I.J.K.S.: acquisition of data and revision of manuscript for critical intellectual content.
N.E.M.: acquisition of data and revision of manuscript for critical intellectual content.
S.J.I.: acquisition of data and revision of manuscript for critical intellectual content.
M.A.K.: acquisition of data and revision of manuscript for critical intellectual content.
F.C.: acquisition of data and revision of manuscript for critical intellectual content.
A.M.: acquisition of data and revision of manuscript for critical intellectual content.
J.-M.d.S.A.: acquisition of data, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
V.S.C.F.: acquisition of data, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
M.V.: acquisition of data, phenotype of family IV from disease onset over 20-year follow-up, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
M.B.: study design and concept, study supervision, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
T.M.: study design and concept, study supervision, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
J.W.: study design and concept, study supervision, analysis and interpretation of data, and revision of manuscript for critical intellectual content.
S.B.: study design and concept, study supervision, analysis and interpretation of data, and revision of manuscript for critical intellectual content.

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