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AOPEP variants as a novel cause of recessive dystonia: Generalized dystonia and dystonia-parkinsonism

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ABSTRACT

Introduction: The genetic basis of autosomal-recessive dystonia remains poorly understood. Our objective was to report identification of additional individuals with variants in *AOPEP*, a recently described gene for recessively inherited dystonic disorders (OMIM:619565).
Methods: Ongoing analysis on a high-throughput genetic platform and international case-recruitment efforts were undertaken.
Results: Novel biallelic, likely pathogenic loss-of-function alleles were identified in two pedigrees of different ethnic background. Two members of a consanguineous Iranian family shared a homozygous c.1917-1G>A essential splice-site variant and featured presentations of adolescence-onset generalized dystonia. An individual of Chinese descent, homozygous for the nonsense variant c.1909G>T (p.Glu637*), displayed childhood-onset generalized dystonia combined with later-manifesting parkinsonism. One additional Iranian patient with adolescence-onset generalized dystonia carried an ultrarare, likely protein-damaging homozygous missense variant (c.1201C>T [p.Arg401Trp]).
Conclusions: These findings support the implication of *AOPEP* in recessive forms of generalized dystonia and dystonia-parkinsonism. Biallelic *AOPEP* variants represent a worldwide cause of dystonic movement-disorder phenotypes and should be considered in dystonia molecular testing approaches.

1. Introduction

Classic monogenic subtypes of isolated dystonia can be inherited in an autosomal-dominant, autosomal-recessive, or X-linked fashion [1]. Autosomal-recessive transmission modes have been well established for variants in *HPCA* (OMIM*142622) [2,3] and *PRKRA* (OMIM*603424)

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[4,5], although the associated conditions have been shown to frequently involve additional non-dystonic neurological features such as intellectual impairment (*HPCA*) [6,7] and parkinsonism (*PRKRA*) [8]. Very recently, our group has uncovered in dystonic patients homozygous and compound heterozygous nonsense, frameshift, and splice-site variants in *AOPEP* (OMIM*619600), a gene with vastly uncharacterized (patho-) physiological functions [9]. The *AOPEP*-related disorder, now referred to as dystonia 31 (DYT31) or Zech-Boesch syndrome in the OMIM database [10], was characterized by generalized or multifocal isolated dystonia of varying onset in 3 families and dystonia coexisting with Parkinsonian signs in a 4th pedigree [9]. It has not yet been confirmed whether the latter association was coincidental or part of a broader *AOPEP*-associated clinical disease spectrum. An additional singular case with generalized isolated dystonia and a homozygous duplication variant in *AOPEP* has been published by Fevga and colleagues [11]. The role of *AOPEP* variants needs to be studied further in independent populations of different geographical origin (see Table 1, Fig. 1).

We report here 4 newly identified affected individuals from 2 Iranian families (3 patients) and a Chinese family (1 patient) with autosomal-recessive *AOPEP*-related dystonia, providing additional supportive evidence for an etiological involvement of *AOPEP* biallelic variants in both generalized dystonia and dystonia-parkinsonism phenotypes.

2. Methods

2.1. Patient recruitment

Direct communications via a network of collaborators with interest in rare movement disorders allowed the identification of these 3 new families. The patients and their healthy family members were enrolled with written informed consent and local ethics review board approvals. Family-1 and family-3 were recruited for genetic analysis through programs dedicated to the discovery of molecular disease etiologies in consanguineous marriages from Iran (University of Isfahan and University of Mashhad, Iran). Family-2 was included in a multi-center study of suspected monogenic dystonias at Technical University of Munich and Helmholtz Center Munich (Munich, Germany) [12]; the affected individual of this family was part of a cohort of 70 index patients who had undergone panel screening without detection of etiological variants in well-established dystonia-related genes (Besta Institute, Milan, Italy) [13] and whose genetic testing was expanded to whole-exome sequencing (WES). Systematic longitudinal phenotype data were obtained for all 4 affected patients by review of chart records, information from treating physicians, and results of standardized clinical examination protocols.

2.2. High-throughput genetic screening and variant confirmation

Exome-library preparation, sequencing, and analysis of DNA isolated

Table 1
Biallelic *AOPEP* variants in families from Iran and China.

	Family 1		Family 2	Family 3
	VI.2	VI.1	III.6	IV.1
Genetic variants				
AOPEP variant	c.1917-1G>A (p.?)	c.1917-1G>A (p.?)	c.1909G>T (p.Glu637*)	c.1201C>T (p.Arg401Trp)
Variant zygosity	homozygous	homozygous	homozygous	homozygous
Variant type	loss-of-function variant	loss-of-function variant	loss-of-function variant	missense variant
Variant frequency (controls)	gnomAD, in-house controls, Iranome: not found	gnomAD, in-house controls, Iranome: not found	gnomAD: 7 heterozygotes; in-house controls: not found	gnomAD: 14 heterozygotes; in-house controls: 9 heterozygotes; Iranome: not found
CADD prediction (score)	25	25	42	29
ACMG classification	(likely) pathogenic[a]	(likely) pathogenic[a]	(likely) pathogenic[b]	variant of uncertain significance (favoring likely pathogenic)[a]
Demographics and family information				
Age	25 years	20 years	34 years	31 years
Gender	male	male	male	male
Geographical origin (ethnicity)	Iran	Iran	China	Iran
Family history	positive (2 affected cousins)	positive (2 affected cousins)	negative	negative
Consanguinity	yes	yes	unknown	yes
Dystonia characteristics				
Age at onset	18 years	13 years	6 years	20 years
Site of onset	arm	arm	arm	arm/neck
Body distribution (most recent examination)	generalized	generalized	generalized	generalized
Involved areas (most recent examination)	upper limbs, lower limbs, trunk, craniocervical region	upper limbs, lower limbs, trunk, craniocervical region	upper limbs, lower limbs, trunk, craniocervical region	upper limbs, lower limbs, trunk, craniocervical region
Tremor	no	no	yes (arm)	no
Associated features				
Other movement disorders	no	no	parkinsonism	no
Other neurological features	no	no	intellectual and behavioral problems[c]	no
Systemic features	no	no	macrocephaly[c]	no
Additional clinical information				
Brain MRI abnormality	no	no	no	no
Treatment attempts	anticholinergics	–	levodopa, left thalamic DBS, bilateral GPi-DBS, anticholinergics, gabapentine, duloxetine, botulinum toxin	–

[a] No additional pathogenic or likely pathogenic variants could be identified in any OMIM-annotated genes known for dystonia.
[b] An additional heterozygous mosaic *CHD8* likely pathogenic variant (c.3725G>A [p.Arg1242Gln], ClinVar-Accession:VCV001178331.1) was observed in the patient`s exome.
[c] These coexisting symptoms may be related to the detected *CHD8* likely pathogenic variant.

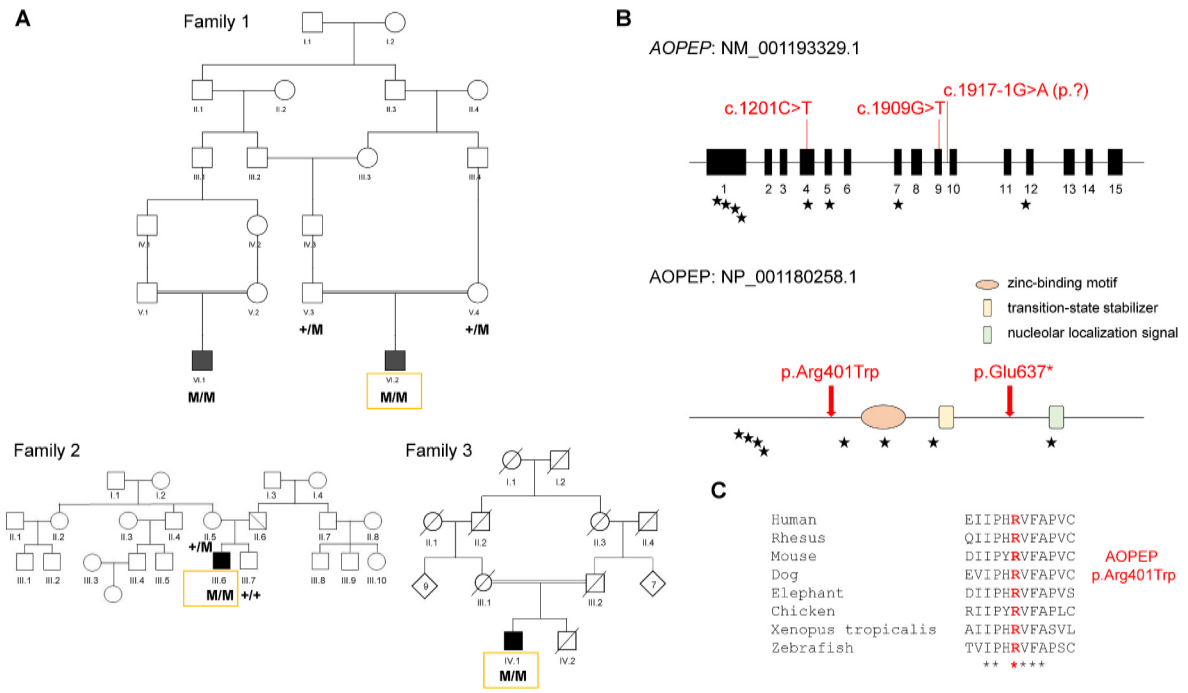

Fig. 1. Biallelic *AOPEP* variants in three new families with dystonia. (A) Pedigrees of families with homozygous *AOPEP* variants. M/M indicates a homozygous *AOPEP* mutation status; +/M indicates a heterozygous carrier status for the familial *AOPEP* variant; +/+ indicates a homozygous wild-type carrier. Index patients in the three families who underwent exome sequencing are highlighted with orange boxes. (B) Locations of the newly identified *AOPEP* variants on the gene level and corresponding protein. Black asterisks indicate the locations of biallelic loss-of-function variants associated with dystonia in our previous gene-identification study [9] and in a study by Fevga et al. [11]. (C) Multiple alignment of AOPEP orthologs demonstrating a high degree of conservation of the residue affected by the herein described homozygous missense variant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

from blood of the patients and their unaffected relatives were conducted using previously reported standard methods [12,14]. Briefly, DNA from patient VI.2 (family-1), patient III.6 (family-2), and patient IV.1 (family-3) was enriched in exonic and adjacent intronic sequences using in-solution capture kits and run on Illumina sequencing machines. Sequence-quality evaluation, alignment, and variant identification and annotation were performed using default parameters as described [12,14]. We assessed the data assuming high penetrance, allowing for the possibility of all transmission modes but focusing on homozygous alleles in light of the observed pedigree structures. Frequency filtering employed a 0.5% threshold based on information from gnomAD [15] and in-house genotype data, including the Iranome for families 1 and 3 [16]. Variants in *AOPEP* (NM_001193329.1) and *CHD8* (NM_001170629.2) were verified by Sanger analysis and tested for cosegregation in available family members.

3. Results

3.1. Family-1

In VI.2, WES uncovered a homozygous *AOPEP* c.1917-1G>A canonical splice-site variant. The variant was absent from gnomAD and local exome databases [15–17]. The exon-10 splice-acceptor dinucleotide affected by c.1917-1G>A was invariant across species [18], and the variation predicted to be deleterious [19]. *In-silico* evaluation using the Variant Effect Predictor tool (Ensembl) [20] suggested a loss-of-function of the authentic acceptor splice-site. Sanger sequencing confirmed the homozygous variant in VI.2 and its perfect co-segregation with disease, revealing that VI.2`s affected cousin (VI.1) was also homozygous for the mutation while VI.2`s healthy parents were each heterozygous carriers.

Cousins VI.2 and VI.1 had highly similar phenotypic presentations. After uneventful pregnancies, both patients were born at full term. They displayed normal psychomotor status until adolescence, when both individuals started experiencing gradual onset of involuntary movements in their extremities. VI.2 first noted impaired voluntary actions of the right hand/forearm as a result of sustained spasms at the age of 18 years; he manifested dystonic wrist flexion and hyperextension of the fingers on outstretched arms bilaterally. Contractions involving the lower limbs soon followed, with plantarflexion posturing of both ankles and leg rotation. He also developed speech impairment, orofacial dyskinesia, and unnatural trunk and neck postures. VI.1 presented at the age of 13 years with occasional patterned flexion of the right-hand fingers; these symptoms continued over the ensuing 3 years before progressive worsening was observed, with appearance of 4-limb dystonia and laterotorticollis. He also experienced articulation deficits, abnormal facial movements, and dystonic trunk deviation. On recent examination, VI.2 (25 years old) and VI.1 (20 years old) displayed generalized isolated dystonia with involvement of the torso, cranio-cervical districts, and all 4 limbs. Blood laboratory studies and brain MRI gave normal results. The family was of Iranian ancestry.

3.2. Family-2

III.6 was identified by WES to carry a homozygous c.1909G>T (p.Glu637*) nonsense variant in *AOPEP*. Populational data showed that c.1909G>T had an extremely low frequency (no homozygous carriers found in controls) [15,17]. The variant was predicted to produce an early stop in exon-9 and premature protein truncation. Sanger sequencing validated homozygosity for the variant in III.6 and demonstrated that the healthy mother was a heterozygous carrier; his healthy brother showed homozygous wild-type sequence. We considered the *AOPEP* variation the most likely cause of III.6`s phenotype, although an additional heterozygous mosaic *CHD8* likely pathogenic variant (c.3725G>A [p.Arg1242Gln]) [21] was observed in the patient`s exome; this *CHD8* alteration was seen in 9/49 (18%) of next-generation sequencing reads, and visualized with significantly reduced peak height in confirmatory Sanger analysis (data not shown), compatible with low-level mosaicism.

III.6 was born via spontaneous delivery and the postnatal course was uncomplicated. He had been reportedly well until he was 6 years of age, when he began to express dystonic posturing in his right arm associated with dystonic tremor. His motor status deteriorated over the following 5 years when he progressively developed movement abnormalities in his right foot, trunk, and neck, consistent with a diagnosis of generalized dystonia. He was also noted to have hypokinetic-dystonic dysarthria, eyelid-opening dystonic "apraxia", cognitive impairment, anxiety, and macrocephaly. Due to dystonia severity, the patient underwent unsuccessful left-thalamic DBS (19 years), followed by bilateral GPi-DBS (20 years) which led to improvement of axial and limb action-dystonia (preoperative BFDRS-M = 65/120, 13-years-follow-up BFMDRS-M = 40). By age 22, he demonstrated symmetric limb akinesia, poor postural reflexes, and worsening gait disorder with start hesitation and freezing resulting in preferential use of a wheelchair. These symptoms were nonresponsive to levodopa, and did not improve after DBS switching-off. On last examination (34 years old), the patient exhibited generalized dystonia combined with prominent akinetic axial parkinsonism. Brain MRI was normal and routine tests provided no evidence for a metabolic disorder. The patient's parents were of Chinese origin.

3.3. Family-3

A homozygous *AOPEP* c.1201C>T (p.Arg401Trp) missense variant was detected via WES in IV.1 as the most likely causative genetic factor. The variant was extremely rare in control cohorts (no homozygotes documented) [15–17], affected a strictly conserved amino-acid position [18], and bioinformatics tools consistently predicted its deleterious nature [19,22,23]. The homozygous substitution was Sanger-verified in IV.1.

IV.1 had an uneventful peri- and postnatal history; early development was normal. By age 20 years, he began to require assistance in daily activities due to painful arm spasms and involuntary neck posturing. He also developed sustained abnormalities of posture in his distal legs. Neurologic assessment completed at 31 years of age was remarkable for generalized dystonia with trunk, neck, and 4-limb involvement (more prominent on the left side) as well as dysarthric speech. The remainder of the examination was normal, as were neuroimaging and metabolic screenings. IV.1 was the offspring of an Iranian family.

4. Discussion

Establishing the clinical validity of gene-disease relationships is an important prerequisite for providing patients with accurate diagnoses and tailored counseling [24,25]. In the case of recessive dystonias, locus confirmation has often proven difficult because of the rarity of the individual conditions [26] and a lack of implementation of genome-wide screening approaches in large patient collections [27]. Our report on homozygous likely-deleterious variants in 4 additional patients with clinical phenotypes similar to those of the previously described *AOPEP*-related dystonia-affected individuals [9] adds strong confirmatory evidence for an association between *AOPEP* and recessive dystonia. We identified predicted loss-of-function alleles in 2 families which could be readily categorized as (likely) pathogenic variants according to the guidelines proposed by the American College of Medical Genetics and Genomics [28]. In a 3rd pedigree, we found a homozygous missense variant, a mutation type not previously implicated in *AOPEP*-related disease [9]. Although this genotype was formally classified as of uncertain clinical significance [28], we considered c.1201C>T (p.Arg401Trp) to be causally related to our patient`s condition based on the mutation occurring at an extremely conserved residue, with deleterious *in-silico* predictions and very rare population representation, and the goodness of fit of the observed presentation to the emerging *AOPEP*-related dystonia phenotype [9]. Our comparatively rapid discovery of additional independent families with biallelic *AOPEP* variants (<6 months after publication of the gene-identification study [9]) suggests that *AOPEP*-related dystonia may be a more common form of hereditary dystonia, with overrepresentation in consanguineous populations. *AOPEP*-related dystonia-affected individuals have now been reported in Australia, China, France, Germany, India, Italy, and Iran, underlining the benefit of community data-sharing across continents for characterization of new gene-phenotype links [9,11].

Previously reported patients with *AOPEP* variants displayed relative phenotypical homogeneity with childhood-to-early adulthood-onset generalized or multifocal dystonia and no significant developmental comorbidity (normal neurodevelopment and intellect in all cases) [9,11]. In accordance with these characteristics, the patients in our families 1 and 3 presented with DYT1-or DYT6-like pictures of early-onset generalized isolated dystonia. Additionally, we observed later-onset, co-manifesting dopa-unresponsive parkinsonism in the affected individual of family 2, which has also been documented in 1 previously published family [9]. This finding supports a role for *AOPEP* perturbation in the etiology of (co-occurring) Parkinsonian features, and contributes to a further expansion of the locus heterogeneity in dystonia-parkinsonism syndromes [29]. III.6`s more complex phenotype with mild dysmorphia and intellectual problems may also be due to his additional *CHD8* variant which could act as an independent or additive disease-contributory lesion [30].

We conclude that biallelic *AOPEP* variants represent an important additional cause of dystonia that is inherited in an autosomal-recessive fashion. Future studies are warranted to determine the frequency of *AOPEP* mutations in cohorts of patients with dystonia-parkinsonism and to further understand the role of missense variation in this gene in the etiology of dystonic phenotypes.

Declaration of competing interest

None of the authors report disclosures concerning the present manuscript.

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