Movement Disorders
CLINICAL PRACTICE

CASE SERIES WITH LITERATURE REVIEW

# The Clinical Spectrum of *ANO3*—Report of a New Family and Literature Review

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**Abstract:** Background: Mutations in *ANO3* are a rare cause of autosomal dominant isolated or combined dystonia, mainly presenting in adulthood.

Cases: We extensively characterize a new, large *ANO3* family with six affected carriers. The proband is a young girl who had suffered from tremor and painful dystonic movements in her right arm since the age of 11 years. She later developed a diffuse dystonic tremor and mild extrapyramidal signs (ie, rigidity and hypodiadochokinesis) in her right arm. She also suffered from psychomotor delay and learning difficulties. Repeated structural and functional neuroimaging were unremarkable. A dystonic tremor was also present in her two sisters. Her paternal aunt, father, and a third older sister presented episodic postural tremor in the arms. The father and one sister also presented learning difficulties. The heterozygous p.G6V variant in *ANO3* was identified in all affected subjects.

Literature review: Stratification by age at onset divided *ANO3* cases into two major groups, where younger patients displayed a more severe phenotype, probably due to variants near the scrambling domain.

Conclusions: We describe the phenotype of a new *ANO3* family and highlight the need for functional studies to explore the impact of *ANO3* variants on its phospholipid scrambling activity.

## Abbreviations

| | |
|---|---|
| AaO | age at onset |
| DBS-GPi | deep brain stimulation of the globus pallidus interna |
| IQ | intelligence quotient |
| NGS | next-generation sequencing |
| WAIS-R | Wechsler's Adult Intelligence Scale-Revised |

Since the advent of next-generation sequencing (NGS), several genes have been associated with dystonic forms with a highly variable inheritance pattern, ranging from isolated forms of dystonia to complex dystonic syndromes.[1] Mutations in *ANO3* are a rare cause of autosomal dominant isolated or combined dystonia, mainly presenting in adulthood with cranio-cervical involvement, with or without dystonic tremor. Since the first report by Charlesworth et al. in 2012,[2] many different phenotypes have been described, from adult-onset focal dystonia to combined generalized dystonia with onset during the first months of life.[3,4]

*ANO3* encodes for anoctamin-3, also known as TMEM16C, a member of the TMEM16 protein family. TMEM16C is a calcium-activated phospholipid scramblase localized to the plasma membrane that is mainly expressed in the brain, particularly in the striatum.[2] The putative role of TMEM16C in neuronal cells is still unknown.

In this report, we describe an Italian family with a variant in *ANO3* presenting with learning difficulties and dystonic tremor and showing intrafamilial variability. We also performed a literature review of *ANO3* cases to attempt a genotype–phenotype correlation.

## Case Series

A summary of demographic and clinical data of all examined family members is presented in Table 1. The family tree is shown in Figure 1.

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## Case 1 (III.1)

A 21-year-old girl sought medical attention for the presence of postural and rest tremor in her right hand since the age of 11 years, enhanced by emotional stress. Action tremor and learning difficulties recurred in her family. During childhood, she also presented delayed psychomotor milestones and was diagnosed with dyslexia, dysgraphia, and dyscalculia. At 16 years, a few months later an orthopedic surgery on her right hand, she observed a worsening of her tremor; this was initially diagnosed as essential tremor and treated with topiramate, propranolol, and carbamazepine, without clear benefit. Two years later, she developed painful dystonic movements in her neck and right arm, mainly after physical exertion, impeding her ability to write and eat independently. She underwent 123I-FP-CIT SPECT, which showed low dopamine transporter binding values in the striatum bilaterally, but still within the normal range. A trial with levodopa (L-dopa) (up to 400 mg/day) and safinamide (up to 100 mg/day) was attempted, with partial benefit on the tremor and dystonic postures, allowing her to resume writing and eating. A few months after starting L-dopa, the tremor spread to her lower limbs, interfering with walking and running. Due to clinical worsening, she was admitted to the Parkinson Institute of Milan for treatment optimization and diagnostic workup.

An extensive neurological examination revealed postural tremor in both hands, tremor in her lower limbs while standing but not while walking or running, rigidity of her neck and right limbs, right hypodiadochokinesis, reduced deep tendon reflexes to the right, reduced arm swinging to the right, a rigid arm posture during walking, unsteadiness, wide-based gait, and impaired tandem gait (Video 1). Other findings were unremarkable. The 100-mg L-dopa challenge did not show a clear clinical improvement after 90 minutes, despite subjective clinical benefit. Electromyography (EMG) showed bilateral postural tremor in her arms, more evident to the right (9 vs. 7 Hz), characterized by the alternating contraction of agonists and antagonists. An 11-Hz lower-limb tremor while standing, characterized by co-contractions of agonists and antagonists, was documented. She obtained low scores on the Wechsler's Adult Intelligence Scale-Revised (WAIS-R)[5] (Table 1). All instrumental examinations were unremarkable (Table 1).

Within a year of hospitalization, she developed spasms in her limbs and the right part of her face, mandibular tremor, and swallowing difficulties. Neurological examination showed symmetric bilateral resting and postural tremor in her hands, mild resting tremor in her left feet, tremor in her lower limbs while standing, cervical dystonia, moderate rigidity of her neck and lower limbs, moderate bradykinesia in all limbs, and symmetric reduction of deep tendon reflexes, wide-based gait, and unsteadiness (Video 2). Repeated EMG confirmed the presence of a diffuse dystonic tremor, with prevalence on her lower limbs.

**TABLE 1** *Demographic and clinical data of ANO3 carriers reported in this study*

| | III.1 (proband) | II.2 | III.3 | III.5 | III.4 | II.1 |
|---|---|---|---|---|---|---|
| Gender | F | M | F | F | F | F |
| Age at diagnosis (yr) | 21 | 49 | 19 | 20 | 24 | 56 |
| Age at onset (yr) | 11 | – | 16 | 20 | – | 55 |
| Psychomotor delay | Yes | No | No | Yes | No | No |
| Learning difficulties | Yes | Yes | No | Yes | No | No |
| WAIS-R scale (total IQ) [85–115] | 71 | 94 | 111 | 75 | 88 | 107 |
| Motor symptoms | Resting and postural tremor<br>Generalized dystonia, tremulous (mainly lower limbs)<br>Parkinsonism | Episodic postural tremor, with cramps | Postural tremor<br>Multifocal dystonia | Postural tremor | Episodic postural tremor | Postural tremor |
| Site at onset | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb | Upper limb |
| EMG | Dystonic pattern | Normal | Dystonic pattern | Dystonic pattern | Not available | Irregular postural tremor |
| Brain MRI | Normal | Normal | Normal | Normal | Normal | Normal |
| 18-FDG PET | Normal | Normal | Normal | Normal | Normal | Normal |
| DaTSCAN | Normal | Normal | Normal | Normal | Normal | Normal |

Abbreviations: F, female; M, male; WAIS-R, Wechsler's Adult Intelligence Scale-Revised; IQ, intelligence quotient; EMG, electromyography; MRI, magnetic resonance imaging.

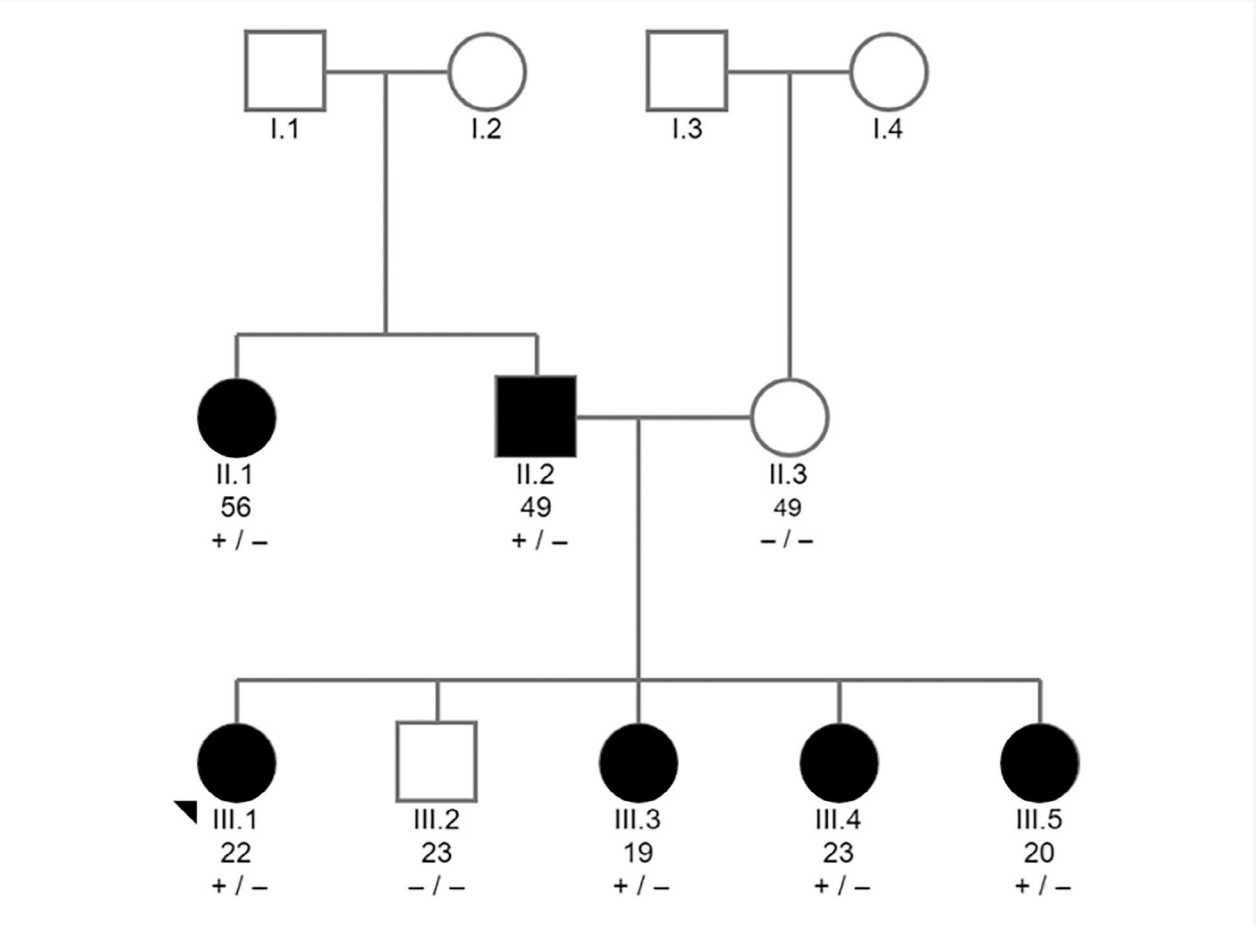



FIG. 1. Family tree. Squares represent males, and circles represent females. White squares or circles represent unaffected family members, whereas black squares or circles represent affected family members. Genetic status is reported under each tested subject: –/–, healthy subject; +/–, heterozygous carrier. The arrow indicates the proband (III.1). WT, wild type.

## Case 2 (III.3)

Her younger sister complained of postural tremor in her right hand, painful irregular movements of her lower limbs during leg extension, and difficulty when walking or running since the age of 16. Two years later, she developed cervical pain and headache, partially responsive to acetaminophen, and writer's cramp in her right hand. Neurological examination confirmed the presence of postural and kinetic tremor in her right hand and action tremor in her left leg during leg extension. She presented reduced right-arm swinging when walking. EMG showed a diffuse dystonic pattern (Table 1).

## Case 3 (III.5)

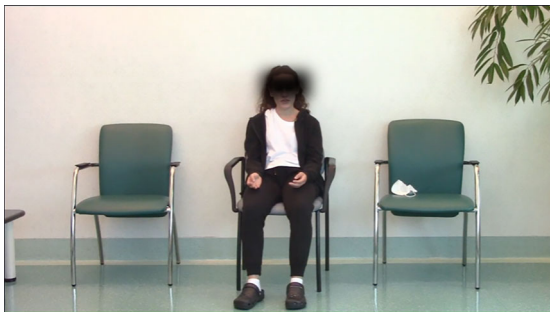
A second younger sister presented delayed developmental milestones, gait difficulties at 13 months, and poor vocabulary. She was later diagnosed with dyslexia and dysgraphia. At the age of 20 years, she observed bilateral postural tremor in her hands after prolonged efforts (ie, carrying weights), as well as pain in her lower limbs. Neurological examination showed only postural tremor in her left lower limb. EMG demonstrated a dystonic pattern at the upper extremities. She obtained low scores on the WAIS-R (Table 1).

## Cases 4 to 6 (II.2, III.4, and II.1)

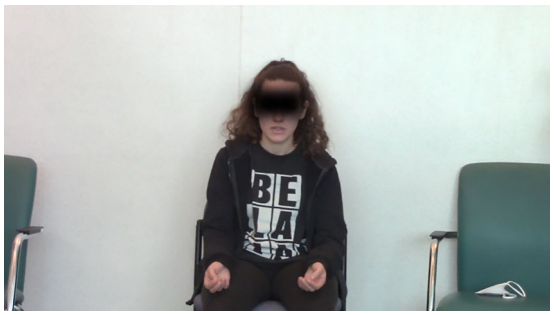
The 21-year-old's father (II.2), older sister (III.4), and paternal aunt (II.1) presented with a milder phenotype characterized by episodic postural tremor of one upper limb, mainly after exertion (II.2 and III.4), or irregular postural tremor of the right upper limb (II.1). Moreover, the father presented learning difficulties, with assistance required during school years.

## Genetic Analysis

After obtaining written informed consent, genomic DNA was extracted from total peripheral blood. An NGS panel, including genes associated with dystonia, was performed (Appendix 1). A very rare heterozygous missense variant in the exon 1 of *ANO3* (NM_031418.4), c.17G>T p.G6V, was identified in all affected subjects and was validated by Sanger sequencing.



**Video 1.** Neurological examination of case III.1 (proband). The video shows a rapid, low-amplitude bilateral action tremor of the upper limbs, slow alternated movements of both hands without clear decrement in amplitude, dystonic postures of right hand and foot, wide-based gait, unstable tandem gait, oscillations of the trunk, and rapid low-amplitude postural tremor of the right lower limb while standing.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13979



**Video 2.** One-year follow-up of case III.1. Neurological examination reveals a rapid, low-amplitude bilateral action tremor of the upper limbs, intermittent head tilting to the right, oscillations of the trunk while standing, dystonic posture of the right hand, wide-based gait, and unsteadiness.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13979

This variant was already present in one subject of European non-Finnish descent on gnomAD v2.1.1 (allele frequency 0.00000879, https://gnomad.broadinstitute.org/) and was reported on ClinVar (RCV001893204, https://www.ncbi.nlm.nih.gov/clinvar/), but was never associated with a phenotype. The variant was classified as likely pathogenic (PP1 strong, PP4 supporting, and PM2 supporting) according to the latest American College of Medical Genetics and Genomics criteria.[6]

# Literature Review

Twenty-five articles were identified[2–4,7–28] (Appendixes 1 and 2). In total, 87 *ANO3* cases were reported (Appendix 2), all heterozygous carriers. Three carriers were asymptomatic,[10,21,26] whereas 6 patients did not show dystonic signs at neurologic examination.[17,26,28] Interestingly, Olschewski et al. reported 2 unrelated cases presenting only parkinsonism.[17] The median age at onset (AaO) was 21 ± 21 years, with two different peaks during childhood and late adulthood (Fig. 2). Focal dystonia was the most common presentation (53/78, 68%), preferentially involving the cervical area and upper or lower limbs. Of note, 2 patients presented with laryngeal involvement only at onset.[2,8] In 42 cases, the dystonic distribution stayed focal or segmental (42/78, 54%). In 15 cases, a generalized pattern was reported (15/78, 19%), mostly following leg involvement at onset. Dystonic tremor was reported in 31 cases (31/78, 40%), whereas almost half displayed nondystonic features such as nondystonic tremor (20/78, 26%), myoclonus (16/78, 21%), and/or L-dopa-responsive parkinsonism (6/78, 8%). When assessed, brain magnetic resonance imaging or computed tomography, 18F-FDG PET, and FP-CIT SPECT were normal. FP-CIT SPECT was pathological in only one case of dystonia parkinsonism, with excellent response to L-dopa.[16] Several therapeutic strategies were reported, with different clinical outcomes. Among medical treatments, anticholinergic drugs and botulinum toxins were the most promising. Clinical relief was achieved with L-dopa mainly when there was association with parkinsonism.[3,16,17,25] Excellent outcomes were obtained with deep brain stimulation of the globus pallidus interna (DBS-GPi).[12,14,17,21]

Stratifying by AaO of dystonia (AaO: ≤20 years, $n = 38$; >20 years, $n = 35$; AaO not reported in 5 cases), younger patients showed preferential leg involvement at onset ($P = 0.003$) even if focal dystonia stayed the most common presentation in both groups ($P = 0.779$) (Table 2). Moreover, younger patients presented a higher probability of generalization (16/38, 42.1%, $P = 0.001$) and need for surgical treatment. Nondystonic features, such as nondystonic tremor ($P = 0.028$), myoclonus ($P = 0.001$), and neurodevelopmental delay, were more prevalent in younger patients. Older patients showed mainly cervical or upper-limb focal dystonia at presentation (15/35, 42.9%, $P = 0.003$), with nearly no tendency toward generalization. Nondystonic features were less represented in older patients ($P = 0.001$), apart from cognitive decline. Stratification according to family history for dystonia is available in Table S2.

A total of 42 *ANO3* variants, mainly missense, were reported (Fig. 3). Seven variants were classified as de novo, occurring only in younger patients[3,4,13,17,18,21,28] ($P = 0.014$). The most common variants were p.A657T and p.S685G, reported in 3 and 4 unrelated families, respectively.[2,8,10,12,16,24,27] *ANO3* variants were widespread along the length of the protein (Fig. S1; Table S1), showing a preference for dimerization and the transmembrane domains 4 and 5. Due to the widespread distribution, a clear genotype–phenotype correlation was difficult to obtain. However, we observed that patients with variants in the transmembrane domains 4 and

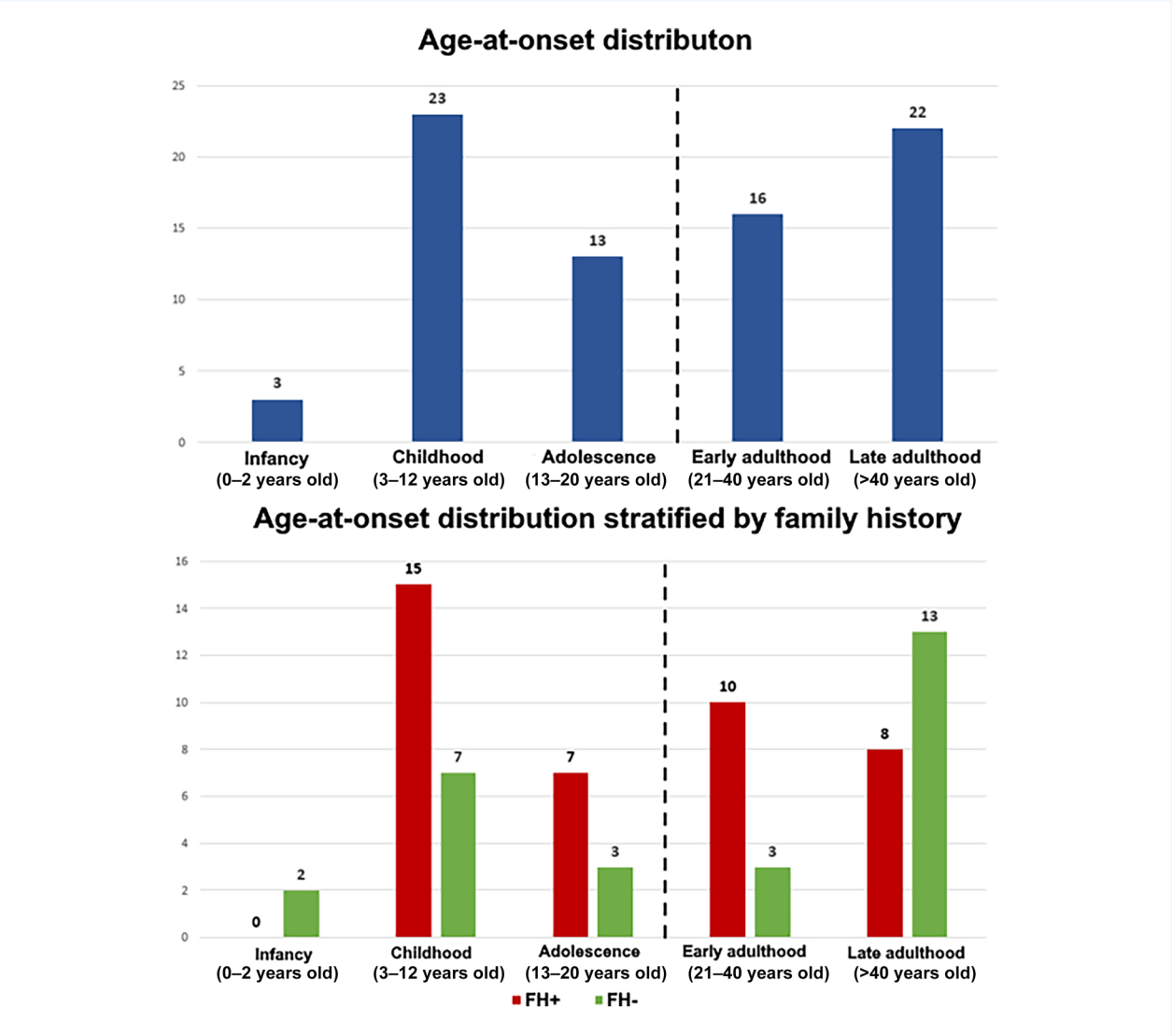



FIG. 2. Age-at-onset distribution. The black dashed line divides the younger group (age at onset ≤20 years) from the older group (age at onset >20 years). FH+ = positive family history for dystonia; FH− = negative family history for dystonia.

5 presented with a higher prevalence of generalized dystonia ($P = 0.001$; Appendix 3).

# Discussion

Our study reports a new *ANO3* family, with 6 affected carriers displaying variable phenotypes, ranging from episodic postural tremor (II.1, II.2, and III.4) to generalized dystonia with parkinsonism (III.1). Three subjects (II.2, III.1, and III.5) presented learning difficulties such as dyslexia or dysgraphia, which has never been associated with *ANO3*. Learning difficulties are common in the general population, so we cannot exclude a possible incidental finding. However, intellectual delay was already reported in 4 previous *ANO3* cases[3,10,13,14,28]; as well, it was associated with other dystonia genes, such as *KMT2B*.[29] The report of episodic postural tremor, mainly after prolonged activities, also seems to be novel in the *ANO3* clinical spectrum. The association between pain and dystonia is common,[30] but the presence of painful dystonic spasms after exertion (III.1 and III.3) was never reported in *ANO3* cases.

The phenotype of the reported family differed among most affected family members, showing a highly intrafamilial variability that has been already reported in *ANO3* families.[25] What is striking is the coexistence of dystonic and non-dystonic phenotypes, with variable degrees of severity, within the same family, which was never reported in previous familial cases. Three family members (II.2, III.4, and II.1) presented with episodic or irregular postural tremor of one of the upper limbs, without clinical or neurophysiological evidence of

**TABLE 2** *Demographic and clinical characteristics of* ANO3 *cases based on age at onset*

| | **AaO ≤ 20 yr (*n* = 39)** | **AaO > 20 yr (*n* = 38)** | |
|---|---|---|---|
| Dystonic patients | 38 (97.4%) | 35 (92.1%) | |
| Nondystonic patients | 1 (2.6%) | 3 (7.9%) | |
| Male[a] | 17 (44.7%) (not reported in 3) | 12 (34.3%) | *P*-value = 0.225 |
| Mean AaO ± SD[ab] | 10.9 ± 5.5 yr | 45.6 ± 13.2 yr | **P-value <0.001** |
| Family history of dystonia[a] | (Not reported in 5) | (Not reported in 5) | *P*-value = 0.180 |
| Positive | 22 (57.9%) | 15 (42.9.5%) | |
| Negative | 11 (28.9%) | 15 (4295%) | |
| Dystonic distribution at onset[a] | (Not reported in 3) | (Not reported in 1) | *P*-value = 0.779 |
| Focal | 25 (65.8%) | 26 (74.3%) | |
| Segmental | 9 (23.7%) | 8 (22.6%) | |
| Multifocal | 1 (2.6%) | 0 (0%) | |
| Sites of focal onset[a] | | | **P-value = 0.003** |
| Cervical | 5 (13.2%) | **15 (42.9%)** | |
| Cranial | 1 (2.6%) | 3 (8.6%) | |
| Laryngeal | 1 (2.6%) | 1 (2.6%) | |
| Lower limbs | **10 (26.3%)** | 0 (0%) | |
| Upper limbs | 8 (21.1%) | 7 (20%) | |
| Evolution pattern of dystonia[a] | | | **P-value = 0.003** |
| Segmental | 7 (18.4%) | 9 (25.7%) | |
| Multifocal | 2 (5.3%) | 2 (5.7%) | |
| Generalization with leg | **12 (31.6%)** | 0 (0%) | |
| Generalization without leg | 2 (5.3%) | 1 (2.6%) | |
| Focal (no evolution) | 5 (13.2%) | **15 (42.9%)** | |
| Segmental (no evolution) | 10 (26.3%) | 8 (22.6%) | |
| Dystonic tremor[a] | 13 (34.2%) | 15 (42.9%) | *P*-value = 0.448 |
| Nondystonic features[c] | | | |
| Nondystonic tremor | **14 (35.9%)** | 5 (13.2%) | **P-value = 0.028** |
| Parkinsonism | 6 (15.4%) | 3 (7.9%) | |
| Chorea | 0 (0%) | 3 (7.9%) | |
| Myoclonus | **14 (35.9%)** | 2 (5.3%) | **P-value = 0.001** |
| Ataxia | 2 (5.1%) | 1 (2.6%) | |
| Tics | 0 (0%) | 1 (2.6%) | |
| Pyramidal signs | 2 (5.1%) | 3 (7.9%) | |
| Hypotonia | 2 (5.1%) | 1 (2.6%) | |
| Psychiatric disorders | 0 (0%) | 3 (7.9%) | |
| Neurodevelopmental delay | **4 (10.3%)** | 0 (0%) | |
| Cognitive decline | 0 (0%) | **4 (10.5%)** | |

*Notes*: Age at onset was not reported in 7 patients, and 3 patients were asymptomatic carriers ($n = 77$). Statistically significant values are reported in bold.
[a]These data refer to the total number of dystonic patients (AaO ≤ 20 years, $n = 38$, AaO > 20 years, $n = 35$).
[b]Quantitative data on age at onset were available for 64 patients.
[c]Nondystonic features were combined in some patients.
Abbreviations: AaO, age at onset; SD, standard deviation.

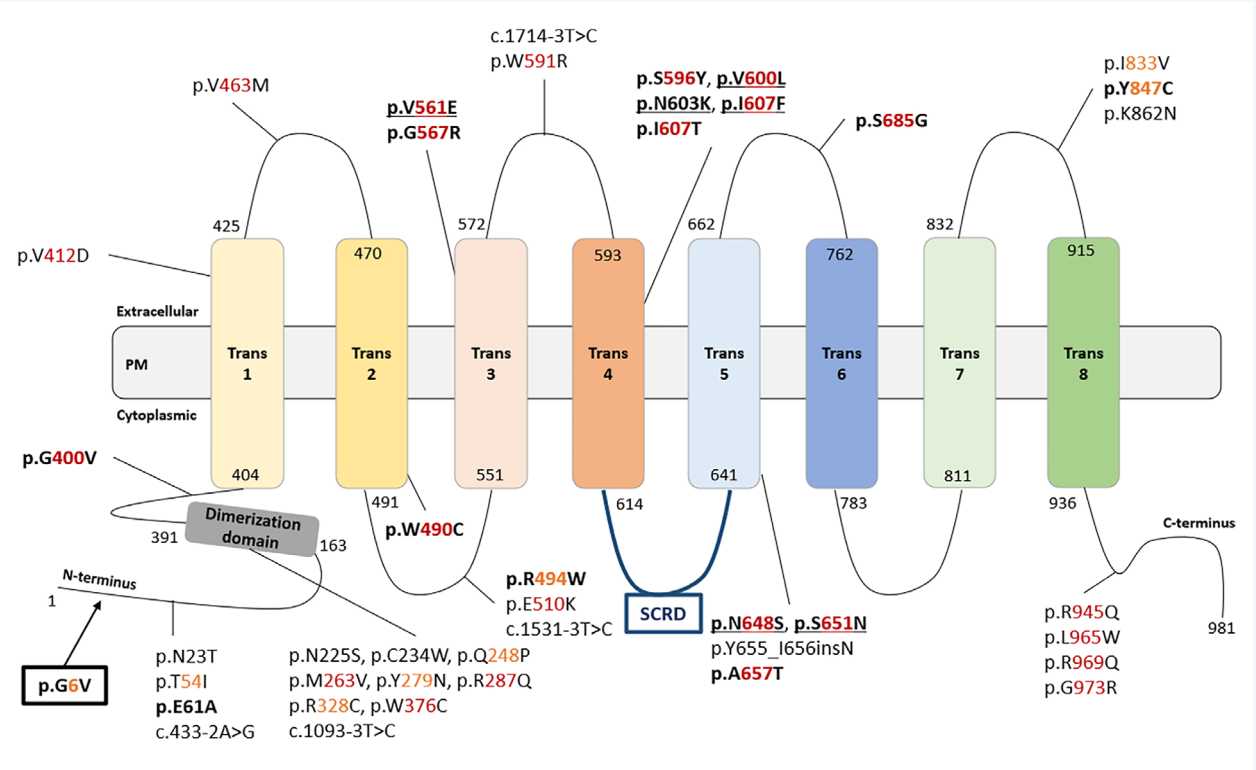



**FIG. 3.** Variant distribution based on *ANO3* protein domains. *ANO3* protein domains are shown based on UniProt data (Q9BYT9-1). Anoctamin-3 presents 8 transmembrane domains, separated by cytoplasmic/extracellular loops. In the N-terminal region resides the dimerization domain. Between transmembrane domains 4 and 5 resides the phospholipid scrambling domain (SCRD). Amino acid residues are colored based on mammalian conservation (orange, moderately; red, highly). Segregating variants are in bold font, whereas de novo variants are in bold font and underlined. The variant reported in this study, p.G6V, resides in the N-terminal region and is shown in a black box. Trans, transmembrane domain.

associated dystonic signs. One could argue against the possibility that all carriers are affected by the same disorder. However, the unilateral involvement, irregular pattern, and emergence of the tremor after prolonged activities or keeping the same position are features that tend toward a possible dystonic origin. Still unknown genetic modifiers and gene–environment interactions could explain this high intrafamilial variability.

In the literature, nondystonic phenotypes were reported in 6 cases. Two unrelated individuals with Parkinson's disease emerged from a large screening of *ANO3* variants in patients with movement disorders. However, the authors did not consider these variants as causative for Parkinson's disease.[17] Santens and colleagues reported a family with different hyperkinetic movements, such as postural tremor, choreic movements, and tics.[26] Finally, Aihara and colleagues reported a de novo case of a young girl with developmental delay, tremor, and ataxic gait.[28] Replications are needed to better define the pathogenic role of *ANO3* in these pure nondystonic phenotypes.

The clinical history of the proband represents a clear example of the difficulties in differential diagnosis between tremulous dystonia and nondystonic tremor, as well as between combined dystonia and early-onset parkinsonism. The latter has been reported in a minority of *ANO3* patients,[3,16,17,25,27] with a good response to L-dopa. Still, FP-CIT SPECT was assessed in only 3 cases,[16,20] with evidence of nigrostriatal degeneration in just 1 case.[16] Regarding the challenging differential diagnosis, NGS technology is useful and guarantees a more precise diagnosis in most cases.

The identified p.G6V variant affects a moderately conserved residue in the N-terminal region. In other proteins belonging to the TMEM16 family, the N-terminal domain is important for dimerization and regulatory mechanisms.[31] Kim et al. showed that the N-terminal region of TMEM16C could be critical for its translocation to the plasma membrane.[32] Functional studies are needed to confirm the potential retention of TMEM16C at inner membranes, due to the variant in the N-terminal region.

The literature review indicates that, in general, the main clinical features of *ANO3* mutations are focal or segmental dystonia of the upper half of the body, with the possibility of dystonic tremor and nondystonic symptoms, with a peak of onset during childhood or late adulthood. However, stratifying by AaO enables *ANO3* cases to be divided into two major groups with distinctive clinical features. Younger patients (≤20 years) presented a more complex phenotype, characterized mainly by a tendency toward generalization, nondystonic tremor, myoclonus, and neurodevelopmental

delay (Table 2). Interestingly, the site of onset for this group was preferentially the lower limbs, a clinical feature that is also typical of other inherited dystonia (eg, *TOR1A*, *KMT2B*, and *GCH1*). The association with myoclonus was also evident in these patients, even if it was not the predominant feature, entering into differential diagnosis with other causes of dystonia myoclonus (eg, *SGCE* with myoclonus > dystonia; *KMT2B, KCTD17* with dystonia > myoclonus). Finally, it is important to also consider a genetic diagnosis in sporadic cases, particularly presenting during youth, due to the possible occurrence of de novo variants.

The identification of the first anoctamins/TMEM16 proteins led to the categorization of the whole protein family as calcium-activated chloride channels.[33] In 2010, Suzuki et al. identified ANO6/TMEM16F as a phospholipid scramblase.[34] Later, the same research team demonstrated that other TMEM16 proteins also presented a phospholipid scrambling activity, including ANO3/TMEM16C.[35] A clear genotype–phenotype correlation was difficult to obtain in our study, but we observed that variants in the transmembrane domains 4 and 5, which are near the scramblase domain (Fig. 3), are associated with a more severe phenotype and tendency toward generalization (Appendix 3). To our knowledge, the impact of known *ANO3* mutations on phospholipid scrambling activity has not been assessed yet. Functional studies in ANO5/TMEM16E showed a clear implication of different variants on scrambling activity. Di Zanni and colleagues[36] demonstrated that monoallelic *ANO5* variants that lead to the skeletal disorder gnathodiaphyseal dysplasia, located in the N-terminal region, are associated with a gain-of-function mechanism, whereas biallelic *ANO5* variants that lead to muscular dystrophy, located in the scrambling domain (SCRD), are associated with a loss-of-function mechanism. Similar studies are needed to better understand the role of *ANO3* variants distributed in different domains in establishing its clinical spectrum.

Several medical treatments were reported, with good results for anticholinergic drugs and botulinum toxins, the latter being particularly helpful in focal and segmental cases. When extrapyramidal signs are present, an L-dopa trial can be attempted, with good outcomes even in cases of normal FP-CIT SPECT. Even if numbers are limited, the use of DBS-GPi showed excellent outcomes in patients with generalized dystonia.[12,14,17,21]

In conclusion, we report a new, well-characterized *ANO3* family displaying variable phenotypes and demonstrated the utility of NGS technology in the differential diagnosis of combined dystonia with tremor and early-onset parkinsonism. A critical literature review showed that variants near the SCRD are associated with a more complex phenotype, but functional studies are needed to confirm this observation.

## Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript: A. Writing of the first draft, B. Review and critique.

M.P.: 1A, 1B, 1C, 3A
M.Z.: 1A, 1B, 1C, 3B
P.S.: 1C, 3B
F.C.: 1C, 3B
M.F.: 1C, 3B
E.O.: 1C, 3B
A.R.: 1C, 3B
C.F.: 1B, 3B
G.P.: 1B, 3B
B.G.: 1C, 3B
I.U.I.: 1A, 1B, 3B
G.S.: 1A, 1B, 1C, 3B

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# Supporting Information

Supporting information may be found in the online version of this article.

**Appendix 1.** Supplementary methods and results.

Genetic analysis, inclusion and exclusion criteria of literature review, and statistical analysis are extensively explained. Additional results regarding stratification by family history and additional figures and tables are presented in Appendix 1.

**Figure S1.** Variant distribution per age at onset, graphic representation.

**Table S1.** Variant distribution per age at onset based on protein domain.

**Table S2.** Demographic and clinical characteristics of *ANO3* cases based on family history.

**Appendix 2.** Demographic, clinical, and genetic data from reported *ANO3* cases in the literature.

Summary regarding demographic, clinical, and genetic data of previously reported *ANO3* cases in the literature.

**Appendix 3.** Statistical results.

Statistical results are extensively explained in Appendix 3.