Dystonia is a clinically and genetically heterogeneous movement disorder. However, genetic causes of dystonia remain largely unknown in Asian subjects. To address this, we applied an integrated two-step approach that included gene dosage analysis and a next-generation sequencing panel containing 72 known genes causative for dystonia and related movement disorders to 318 Taiwanese patients with isolated or combined dystonia. Whole-genome sequencing was performed for one multiplex family with known genes causative for dystonia and related movement disorders to 318 Taiwanese patients with isolated or combined dystonia. Whole-genome sequencing analysis identified a novel intragenic deletion in OPNH1 in a multiplex family with X-linked dystonia and intellectual delay. Our findings delineate the genetic architecture and clinical spectrum of dystonia-causing pathogenic variants in an Asian population. (J Mol Diagn 2022, 1: 1-12; https://doi.org/10.1016/j.jmoldx.2021.12.003)
of ethnically relevant genetics in patients with dystonia in diverse populations. However, genetic causes of dystonia remain largely unknown in Asian populations.

The current study used an integrative approach, including gene dosage analysis and a targeted next-generation sequencing panel, to elucidate genetic causes and the relationships between genotypes and phenotypes in patients with isolated or combined dystonia in a Taiwanese population. Whole-genome sequencing (WGS) was performed for one multiplex family with no known causative variant from panel analysis.

**Materials and Methods**

**Participants**

A total of 318 unrelated patients with isolated or combined dystonia and 39 of their first-degree relatives, including 21 symptomatic and 18 asymptomatic family members, were recruited from movement disorder clinics in two medical centers between January 2015 and December 2020. Of these 39 relatives, 27 were either one or both of parents of the recruited probands. All participants were evaluated by movement disorder specialists (M.-C.W., Y.-Y.C., M.-Y.L., Y.-F.C., C.-H.T., and C.-H.L.). Patients with secondary dystonia, such as neuroleptic agent—induced tardive dystonia, cerebral palsy, posttraumatic dystonic syndrome, and organic brain lesions with gadolinium contrast enhancement on brain MRIs (including brain tumor, demyelinating lesions, and infectious or autoimmune encephalitis), were excluded.

All patients received a routine laboratory workup, including serum ceruloplasmin, to exclude secondary causes of dystonia. Among the 318 enrolled patients, 66 had an age at onset of <20 years, and 84 had a family history of dystonia. Of these latter, 53 had a family inheritance pattern compatible with autosomal dominant (AD) inheritance, and the remaining 31 patients had an autosomal recessive inheritance pattern with affected siblings or they had at least one other first- or second-degree relative with dystonia. Clinical characteristics were recorded for each participant, including age at dystonia onset (≤20 years, 21 to 40 years, or >40 years), ataxia. For patients with combined dystonia, dystonia would be the main feature albeit with the coexistence of other movement disorder features (myoclonus, Parkinsonism, or ataxia). For patients with combined dystonia, dystonia would be the main feature albeit with the coexistence of other movement disorder features.

All participants provided written informed consent, and the study was approved by the institutional ethics review board of the National Taiwan University Hospital (201808019RINB).

**Genetic Analysis**

The flowchart of the genetic analysis is presented in Figure 1.

Multiplex Ligation-Dependent Probe Amplification (MLPA) DNA was extracted from venous blood by using standard protocols. Large deletions or duplications of common dystonia and Parkinson disease (PD)-causative genes, including TOR1A, THAP1, ATP1A3, PRKRA, GCH1, PRKN, PINK1, DJ-1, ATP13A2, PLA2G6, FBX07, DNAJC6, and LRKK2, were detected by using SALSA multiplex ligation-dependent probe amplification kits (SALSA MLPA Probemix P059-B2, P051-c1/P52-c1; MRC-Holland, Amsterdam, the Netherlands). Relative quantification of implicated exons was performed to confirm a homozygous deletion state.

**Targeted Next-Generation Sequencing Panel**

A custom-designed next-generation sequencing panel, including 72 genes associated with dystonia and related movement disorders (Supplemental Table S1), was performed as previously described. 11 Figure 1 depicts the criteria for identifying causative variants in the affected families, including target enrichment, variant calling, and data filtering. In brief, variants with allele frequencies <5% compared with the public databases from the 1000 Genomes Project, NHLBI-ESP 6500 exome project, dbSNP version 151, gnomAD, and Taiwan Biobank exome database (Taiwan Biobank, https://taiwanview.twbiobank.org.tw/index, in Chinese, last accessed October 31, 2021) (1517 exomes) were selected. Missense variants with PolyPhen-2 scores <0.95 and SIFT scores >0.05 were excluded. Variants that were previously described as disease-causing in the literature were regarded as pathogenic variants. Varsome version 10.0 tool platform (Varsome, https://varsome.com, last accessed October 31, 2021) was applied for the classification of pathogenicity based on the American College of Medical Genetics and Genomics interpretation criteria. 12 Variants meeting the criteria were then subjected to Sanger sequencing to confirm the nucleotide change. Segregation patterns were obtained to determine whether a variant co-segregated with dystonia in the pedigrees. For biallelic substitutions, sequence information from the parents and siblings was obtained to determine if the substitutions were in cis (variants occurring on the same chromosome) or in trans (variants occurring on different chromosomes).

**Whole-Genome Sequencing**

We performed WGS in five family members (two affected individuals and three unaffected members) from one multiplex family with no known causative variants from panel analysis. Sanger sequencing of the targeted causative gene was performed for additional family members in this index family for segregation analysis. Paired-end multiplex libraries were prepared according to the manufacturer’s instructions with an Illumina TruSeq DNA Sample Prep Kit (San Diego, CA) and enriched with the NimbleGen SeqCap EZ Human Exome Library version 3.0 (Roche NimbleGen Inc., Pleasanton, CA). The NimbleGen kit targets 64 Mb,
corresponding to 30,000 genes. Libraries were loaded into Illumina flow cells for cluster generation before producing 100-base read pairs on a HiSeq2000 instrument, following the Illumina protocol. Base-calling and quality control were performed with the Illumina RTA sequence analysis pipeline according to the manufacturer’s instructions. Reads were hard trimmed from the end of the read up to the first base with a quality of at least 10. Reads with at least 40 nucleotides in length were mapped to Human Genome build hg19 by using the Genome Multiol tool v1 Application (GEM mapper, version 3), allowing up to four mismatches. Alignment (.bam) files containing only properly paired, uniquely mapping reads were processed by using Picard tools (broadinstitute.github.io/picard, version 1.110) to add read groups and to remove duplicates. We removed common variants in the population that had minor allele frequencies >1% in dbSNP version 15113 or in the Taiwan Biobank.14 For variants in the coding region, PROVEAN (version 1.1.3),15 SIFT version 2019 (SANS Institute, North Bethesda, MD),16 and PolyPhen-2 version 2 (Harvard University, Cambridge, MA)17 were used to predict the potential impact of the variant on protein structure and function. The American College of Medical Genetics and Genomics interpretation criteria were applied for the classification of pathogenicity.12

Statistical Analysis
Cases were grouped based on their clinical characteristics to compare the pathogenic variant rate among groups. Group comparisons were performed with the independent t-test, χ² test, analysis of variance, or the Fisher exact test, depending on the data distribution and number of comparisons. P < 0.05 was considered significant. All analyses were performed with Stata software (StataCorp LLC, College Station, TX).

Results

Overall Demographic Characteristics

The mean age at onset among the 318 patients with dystonia was 41.6 ± 20.0 years (Table 1). A total of 256 (80.5%) patients presented with isolated dystonia, and the remaining 62 (19.5%) patients had combined dystonia with concomitant myoclonus [n = 17 (27.4%)], Parkinsonism [n = 33 (53.2%)], and ataxia [n = 12 (19.4%)]. For patients with combined dystonia, the dystonia was mostly generalized in distribution. Although some patients (n = 15) had more than one co-morbid non-dystonia movement disorder feature, we categorized them into the predominant feature group.

Genetic Findings

Of the 318 enrolled patients, 40 (12.6%) probands have pathogenic or likely pathogenic variants in known genes causative for dystonia and other movement disorders. None of the probands had de novo pathogenic or likely pathogenic variants. Using target gene capture sequencing, we covered 1197 exons in 72 genes representing a total coding region of 284,531 bp. The average coverage was 145-fold, with 93.1% of sequences having coverage >30-fold and 88.9% >50-fold. Among these patients, we found pathogenic or likely pathogenic variants in SGCE (n = 12 from seven families), TH (n = 4), GCH1 (n = 4 from three families), CACNA1B (n = 3), PRRT2 (n = 3), MRI (n = 2), CIZ1 (Harvard University, Cambridge, MA)17 were used to predict the potential impact of the variant on protein structure and function. The American College of Medical Genetics and Genomics interpretation criteria were applied for the classification of pathogenicity.12

Statistical Analysis
Cases were grouped based on their clinical characteristics to compare the pathogenic variant rate among groups. Group comparisons were performed with the independent t-test, χ² test, analysis of variance, or the Fisher exact test, depending on the data distribution and number of comparisons. P < 0.05 was considered significant. All analyses were performed with Stata software (StataCorp LLC, College Station, TX).

Table 1 Demographic Characteristics of Dystonia Probands Enrolled in the Study

<table>
<thead>
<tr>
<th>Total number of probands (N = 318)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Current age (mean ± SD), y</td>
</tr>
<tr>
<td>Age at onset (mean ± SD), y</td>
</tr>
<tr>
<td>Disease duration (mean ± SD), y</td>
</tr>
<tr>
<td>Clinical presentation</td>
</tr>
<tr>
<td>Isolated dystonia, n (%)</td>
</tr>
<tr>
<td>Focal</td>
</tr>
<tr>
<td>Segmental</td>
</tr>
<tr>
<td>Multifocal</td>
</tr>
<tr>
<td>Hemidystonia</td>
</tr>
<tr>
<td>Generalized</td>
</tr>
<tr>
<td>Combined features, n (%)</td>
</tr>
<tr>
<td>With myoclonus</td>
</tr>
<tr>
<td>With Parkinsonism</td>
</tr>
<tr>
<td>With ataxia</td>
</tr>
<tr>
<td>Family history</td>
</tr>
<tr>
<td>AD inheritance</td>
</tr>
<tr>
<td>AR inheritance</td>
</tr>
<tr>
<td>No family history</td>
</tr>
<tr>
<td>AD, autosomal dominant; AR, autosomal recessive; SD, standard deviation.</td>
</tr>
</tbody>
</table>

Table 2 shows the results of the allele frequency analysis for the targeted genes.

Figure 1 Work flow for identifying causative variants in patients with isolated or combined dystonia in this study. BWA, Burrows Wheeler Aligner; GATK, Genome Analysis Toolkit; IGV, Integrative Genomics Viewer; MLPA, multiplex ligation-dependent probe amplification; NGS, next-generation sequencing.
Figure 2 Results of genetic analyses in the current dystonia cohort. A: Proportions and numbers of individuals with known pathogenic or likely pathogenic variants in causative genes. B: Proportions of known pathogenic or likely pathogenic variants according to age categories at onset. C: Proportions of known pathogenic or likely pathogenic variants according to clinical categories, encompassing 229 (isolated dystonia) and 89 (combined dystonia) individuals. D: Proportions of known pathogenic or likely pathogenic variants according to different subgroups in 89 patients with combined dystonia, combined with tremor (n = 27), myoclonus (n = 17), Parkinsonism (n = 33), or ataxia (n = 12). The pathogenic substitution frequency was highest with dystonia combined with myoclonus compared with the combination with tremor (P = 0.005) or Parkinsonism or ataxia (P = 0.04). *P < 0.05. VUS, variants of uncertain significance.

Clinical and Genetic Analysis in Multiple-Case Pathogenic Variant Carriers

Twelve patients from seven families had heterozygous SGCE pathogenic or likely pathogenic substitutions (Supplemental Figure S1). Five had heterozygous variants that were previously reported to be pathogenic, including two with p.G428R missense variant,\(^1\) two with stop-gain substitutions (p.R97X and p.R237X, respectively),\(^{19,20}\) and one with heterozygous deletion from exons 2 to 11.\(^2\) One patient presenting with isolated cranial dystonia had a likely pathogenic heterozygous variant, c.409C>T (p.R137C). The remaining patient with an autosomal recessive inheritance family history of dystonia had a novel heterozygous frameshift insertion in SGCE, c.693_694insCC (p.F23RfsX15) (Family DYS042) (Supplemental Figure S1). The proband with this novel frameshift likely pathogenic variant had been diagnosed with attention-deficit hyperactivity disorder at the age of 7 years; left upper limb dystonia combined with myoclonus subsequently developed at age 14 years. His elder sister with the same variant also had similar phenotypes from age 15 years (Table 2). We also identified three probands having heterozygous GCH1 pathogenic or likely pathogenic substitutions. Two had splicing variants, c.541+1G>T and c.626+1G>C, respectively, both of which have been reported as pathogenic splicing variants.\(^{22-24}\) The remaining patient carried a
novel frameshift deletion variant, c.745delA (p.R249fs), which co-segregated within the family (DYS032 family) (Supplemental Figure S2A). The patient with the c.541+1G>T substitution developed generalized dystonia starting from the legs with diurnal changes from age 8 years. Her family had an AD inheritance pattern of dopa-responsive dystonia (DYS032 family) (Supplemental Figure S2A). The patient with the other splicing substitution, c.626+1G>C, had writer’s cramp from age 30 years and experienced a good response to botulinum toxin treatment. Of note, the proband with the novel frameshift deletion substitution, c.745delA (p.R249fs) in GCH1, was a 44-year-old woman who presented with foot dystonia and right-side levodopa-responsive Parkinsonism features since her early thirties. Her younger brother, daughter, and cousins all had dopa-responsive foot dystonia since their childhood (DYS032 family) (Supplemental Figure S2A). We also identified heterozygous TH likely pathogenic variants in four patients, three of whom had known pathogenic stop-gain or missense variants, two with the c.457C>T (p.R153X) and one with the c.686G>A (p.R229H) variant.36 A novel heterozygous stop-gain variant, c.1136C>A (p.S379X) in TH, was found in one patient with spasmodic dysphonia with partial response to levodopa from age 39 years (Supplemental Figure S2B). However, we did not have the opportunity to examine her family members, which hampers any further segregation analysis. In addition, we identified three patients with heterozygous PRRT2 pathogenic or likely pathogenic variants, all of whom presented with paroxysmal kinesigenic dystonia. One patient whose family exhibited an AD inheritance pattern of dystonia and migraine without hemiplegia carried the heterozygous frameshift substitution c.649dupC (p.R217P fxX70), which is a pathogenic variant hotspot for the PRRT2-related paroxysmal disorders.25,27 The other two patients had sporadic paroxysmal kinesigenic dyskinesia, with one also carrying the c.649dupC (p.R217P fxX70) frameshift substitution and the other having the previously reported missense c.G913A (p.G305R) variant.28

Heterozygous missense substitutions in CACNA1B were identified in three patients, one of whom had an AD inheritance pattern for dystonia. The proband having the heterozygous c.6506A>T (p.N2169I) variant presented with childhood onset of segmental dystonia involving the face, neck, and shoulder, associated with myoclonus. His siblings, father, and aunt were reported to have blepharospasm and cervical dystonia. However, we did not have access to DNA samples from these affected family members, which hampered any further segregation analysis. The other two patients represented sporadic cervical dystonia, one carried the heterozygous c.6694C>T (p.V2310M) variant and the other carried the heterozygous c.6928G>A (p.V2310M) variant. These two variants are predicted to be likely pathogenic and were absent from the 1514 Taiwanese control exome database.14 We also identified two patients harboring heterozygous likely pathogenic variants in CIZ1 gene. One patient had a heterozygous deletional frameshift substitution, c.58_81del24 (p.Leu20_Gln27del), which was

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical Characteristics of Multiple-Case Pathogenic or Likely Pathogenic Substitution Carriers in this Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>SGE</td>
</tr>
<tr>
<td>n = 12 from 7 families</td>
<td></td>
</tr>
<tr>
<td>n = 4 from 3 families</td>
<td></td>
</tr>
<tr>
<td>Zygosity</td>
<td>Hetero</td>
</tr>
<tr>
<td>AAO (mean ± SD), y</td>
<td>27.0 ± 15.1</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Dystonia type</td>
<td></td>
</tr>
<tr>
<td>Cranial</td>
<td>+</td>
</tr>
<tr>
<td>Cervical</td>
<td>+</td>
</tr>
<tr>
<td>Upper limb</td>
<td>+</td>
</tr>
<tr>
<td>Lower limb</td>
<td></td>
</tr>
<tr>
<td>Multifocal</td>
<td>+</td>
</tr>
<tr>
<td>Segmental</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td></td>
</tr>
<tr>
<td>Associated symptoms</td>
<td></td>
</tr>
<tr>
<td>Myoclonus</td>
<td>+</td>
</tr>
<tr>
<td>Tremor</td>
<td></td>
</tr>
<tr>
<td>Parkinsonism</td>
<td></td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>AD = 3</td>
</tr>
<tr>
<td></td>
<td>AR = 1</td>
</tr>
</tbody>
</table>

*“−” indicates absence of the referred symptoms. “+” indicates presence of the referred symptoms.

AAO, age at onset; AD, autosomal dominant; AR, autosomal recessive; hetero, heterozygous; SD, standard deviation.
previously reported to be pathogenic. The other patient with generalized dystonia with mild Parkinsonism starting in his late forties carried a likely pathogenic missense variant c.65A>G (p.Q22R), as did his younger brother with similar symptoms.

Of note, we identified several patients having pathogenic or likely pathogenic variants in Parkinsonism-causative genes, including PLA2G6 and PRKN (Table 2). Two unrelated patients had compound heterozygous PLA2G6 variants, one with c.991G>T (p.D331Y)/c.1186C>T(p.Q396X) and the other with a c.991G>T (p.D331Y)/c.1427+1G>T splicing substitution. Both substitutions are located within the PLA2G6 gene and are predicted to result in truncated and possibly dysfunctional protein products. These patients had clinical features consistent with early-onset PD, indicating that such variants can lead to disease expression even in the absence of a founder effect.

Clinical and Genetic Analysis in Single Cases of Pathologic or Likely Pathogenic Variants

Genetic causes were also identified from single cases involving heterozygous single allelic substitutions in TOR1A (c.907_909del, p.303_303del), TUBB4A (c.38C>A, p.P13Q), THAP1 (c.122G>A, p.R41H), ATP1A3 (c.1825 G>A, p.D609N), ANO3 (c.2053A>G, p.S685G), GNAL (c.2272G>A, p.G758R), KMT2B (c.3043C>T, p.R1015X), SLC6A3 (c.1634C>A, p.P545H), ADCY5 (c.139G>T, p.G47W), C19orf12 (c.273_274insA, p.P92Tfs*9), and SPG11 (c.4462_4463del, p.V1488fs) (Supplemental Table S3). One patient had compound heterozygous variants in exon 3 (c.332 T>A, p.L111Q) and exon 5 (concomitant c.1499A>T, p.N500I) of PANK2, and another patient had the compound heterozygous substitutions c.1072C>T (p.Glu358X) and c.496-497del (p.S503-514) in exon 1 of CYP27A1. The clinical characteristics of patients harboring these variants are summarized in Table 3.

The potentially novel heterozygous variant in C19orf12, c.273_274insA (p.P92Tfs*9), was predicted to be pathogenic. This novel, potentially pathogenic variant cosegregated within the proband’s family, showing an AD inheritance for early-onset dystonia, cognitive decline, and ataxia with eye-of-tiger sign on brain MRI, leading to a diagnosis of the AD inheritance form of mitochondrial membrane protein-associated neurodegeneration. Another patient had a compound heterozygous variant in PARK2. Both patients had experienced generalized dystonia, neck retrocollis, and spasmodic dysphonia, combined with cerebellar ataxia, since their early twenties (Table 3). Brain T2-weighted MRI scans of both patients revealed a bilateral abnormally low signal in the globus pallidus with a central high signal, which are neuroradiologic eye-of-tiger signs of neurodegeneration with brain iron accumulation. The patient with the PARK2 variant received deep brain stimulation to the bilateral globus pallidus at age 30 years with good response and remained independent at age 43.
years, whereas the patient with C19orf12 substitutions deteriorated rapidly and died of aspiration pneumonia 5 years after symptom onset. We also identified known compound heterozygous pathogenic variants in the CYP27A1 gene in a sporadic patient who had experienced generalized dystonia combined with ataxia and xanthomas over Achilles tendons since his teenage years, leading to a diagnosis of cerebrotendinous xanthomatosis. These observations suggest that the integrated genetic approach could be used for molecular diagnosis of patients with dystonia who present with complex neurologic features or non-neurologic presentations. Among these single cases, we also identified patients with DYT1 (TOR1A), DYT4 (TUBB4A), DYT6 (THAP1), DYT12 (ATP1A3), DYT24 (AN03), DYT25 (GNAL), and DYT28 (KMT2B). However, these pathogenic or likely pathogenic variant rates were rare in our cohort (<0.5%).

A Novel Intragenic Deletion in OPHN1 Identified via WGS Analysis

A novel intragenic deletion in the oligophrenin 1 (OPHN1) gene was identified by WGS in a family with X-linked dystonia and intellectual delay. WGS was performed on two affected (III-3 and III-8) and three unaffected (II-1, II-2, and III-1) members of the family (Figure 3A). The average fold coverage of the WGS was 30.02, 24.73, 34.57, 29.32, and 34.21 for individuals II-1, II-2, III-3, III-1, and III-8, respectively, with at least 91.33% (90.9% to 91.6%) of the target region covered by at least 10 sequencing reads. After series of filtrations, we identified three heterozygous missense variants [AFF3 c.287C>G (p. P96R), SLC12A4 c.491G>A (p.C164Y), and COLA9A3 c.281C>T (p.P94L)] and a 400-bp deletion in intron 1 of OPHN1 on the X chromosome. All three variants were predicted to be
potentially pathogenic. Among these three candidate variants or deletions, only the heterozygous intron 1 deletion in *OPHN1* on the X chromosome (67545900 to 67546300) segregated with the phenotypes within the family (Figure 3, A and B). The oligophrenin 1 protein is highly expressed in the brain.39 This intron 1 deletion of *OPHN1* was therefore selected as the candidate in this index family. The clinical phenotypes were male sex and a short philtrum, long face, developmental intellectual delay, and variable degrees of foot and hand dystonia since childhood. Brain MRI scans in all affected members in their early twenties revealed diffuse cortical atrophy, ventriculomegaly, and cerebellar atrophy (Figure 4), which were consistent with those described in patients with *OPHN1* pathogenic variants.40 Our patients have the characteristic features associated with *OPHN1* pathogenic substitutions, and, furthermore, the observed dystonia features extend the clinical spectrum of *OPHN1* pathogenic variants.

Discussion

Using an integrated genetic approach, we identified the genetic causes and established the genotype–phenotype relationship for patients with isolated or combined dystonia in a Taiwanese cohort. Our results showed that 12.6% had pathogenic or likely pathogenic variants in known dystonia-related genes. Genetic diagnosis was more likely with juvenile onset compared with adult onset and with combined features, especially with the myoclonus phenotype, in our population. The WGS analysis identified a novel intragenic deletion in *OPHN1* in a multiplex family with X-linked dystonia and intellectual delay.

In keeping with previous findings, our results showed that the genetic diagnostic yield was highest among those with younger age at onset and among patients with generalized dystonia co-existing with other neurologic features.43 In patients with combined dystonia and myoclonus, variants in DYT11 (*SGCE*) were most prevalent, followed by variants in DYT23 (CACNA1B) and DYT24 (ANO3). Among these genetic variants, substitutions in *SGCE* were present in almost 16% of patients with combined dystonia and in 46% with dystonia-myoclonus syndrome. Compared with previous studies in western populations, the pathogenic or likely pathogenic variant frequency of *SGCE* was higher in our study cohort. One study describing whole-exome sequencing for 189 patients from a mixed Caucasian population did not identify any patients with *SGCE* pathogenic variants,6 and another study of patients from Australia and India found that 1.8% of patients carried pathogenic substitutions in *SGCE*.4 In a recent, large mixed European cohort study of 764 patients with dystonia, substitutions in the *SGCE* gene accounted for only 4% of the patients.41 Ethnic differences in *SGCE* genetic variants is one of the reasons contributing to the variations in prevalence. In addition, among the 318 enrolled patients with dystonia, 84 (26.4%) had a family history of dystonia. This high proportion of positive family history in our enrolled participants may be because our institutes are tertiary referral centers for movement disorders, which may have the tendency to enrich the cohort for potentially genetically determined cases.

Regardless of ethnicity-related differences in genetic variant frequency, the phenotypes of those with *SGCE* substitutions are similar, with the dystonia mainly involving the cervical and upper limbs, with varying degrees of myoclonus. In addition, those with pathogenic variants in DYT23 (CACNA1B) and DYT24 (ANO3) can present with cervical dystonia and myoclonus.42,43 In this study, we also identified three likely pathogenic variants in CACNA1B and one reported heterozygous pathogenic variant of ANO3 in patients with cervical dystonia and variable degrees of myoclonus. Taking together, given the clinical heterogeneity and occasional lack of family history, especially for those with *SGCE* substitutions related to maternal genomic imprinting, we suggest that genetic variants in *SGCE*, CACNA1B, and ANO3 should be highly suspected for those with dystonia-myoclonus syndrome in our population.

Of note, in another subgroup of patients with combined dystonia and Parkinsonism, biallelic variants in PLA2G6 and PRKN were the most prevalent, followed by compound heterozygous substitutions in PANK2 and heterozygous variants in DYT5a (GCH), DYT5b (TH), DYT12 (ATP1A3), CIZ1, and C19orf12. PRKN is the most commonly mutated gene in patients with early-onset PD and contributed to 10% of cases with an onset age younger than 40 years in our population.11 Patients with PRKN pathogenic or likely pathogenic variants have foot dystonia as an initial presentation that can sometimes precede Parkinsonism symptoms by years. Variants in PLA2G6 have been associated with a heterogeneous group of neurodegenerative disorders, including infantile neuroaxonal dystrophy, early adult-onset dystonia-Parkinsonism (known as neurodegeneration with brain iron accumulation type II), and autosomal recessive early-onset Parkinsonism (known as PARK14).44 Two patients in our study had compound heterozygous variants in PLA2G6, including c.991 G>T (p.D331Y)/p.Q396X (c.1186 C>T) in one and a c.991 G>T (p.D331Y)/c.1427+1 G>T splicing substitution in the other. This result reinforces our previous findings showing that the c.991G>T (p.D331Y) substitution in *PLA2G6* is found almost exclusively in patients of Chinese ancestry, suggesting a common founder effect of this variant in our population.45 These observations suggest that patients who are homozygous for p.D331Y variants commonly have purely early-onset PD with good levodopa responses, whereas those with compound heterozygous p.D331Y substitutions and other variants, as identified in our two patients, predominantly present with dystonia-Parkinsonism features. In addition, we identified one heterozygous novel frameshift substitution, c.273_274insA (p.P92Tfs*9), in C19orf12. This variant was found in an AD inheritance
family with young-onset dystonia combining Parkinsonism and ataxia symptoms. Pathogenic or likely pathogenic variants within C19orf12 have recently been identified in patients with mitochondrial membrane protein-associated neurodegeneration, which is believed to be the third most frequent subtype of neurodegeneration with brain iron accumulation after PANK2- and PLA2G6-associated neurodegeneration. Although mitochondrial membrane protein-associated neurodegeneration previously has been documented to follow an autosomal recessive pattern of inheritance, recent reports have identified several patients with mitochondrial membrane protein-associated neurodegeneration who were heterozygous for pathogenic variants in C19orf12 segregating in an AD-inheritance pattern, suggesting that a single mutant allele can cause the disease. C19orf12 encodes a mitochondrial membrane protein involved in lipid metabolism, and pathogenic variants in this gene are postulated to cause dysfunction of lipid homeostasis in mitochondria. Because the clinical, pathologic, and neuroradiologic features were almost identical in cases of heterozygous and biallelic substitutions in C19orf12, the heterozygous variants leading to disease may result from a dominant-negative mechanism rather than from gain of function.

We also found that single cases with DYT5a (GCH), DYT5b (TH), and DYT12 (ATP1A3) variants involved dystonia combined with Parkinsonism features. Notably, although biallelic substitutions in TH are required to cause dystonia, four patients with adult-onset dystonia with mild Parkinsonism feature who carried the previously reported pathogenic variants in TH are in the single heterozygous state in our study. In support of our findings, one recent study found that single allelic variant in TH causes adult-onset dopa-responsive dystonia at the age of late thirties. We speculate that, as in some other recessive disorders, heterozygosity in TH may lead to a milder form of generalized dystonia compared with those with biallelic substitutions in TH that typically manifests in the first few years

Figure 4  Brain MRI findings of the affected individuals in the family with OPHN1 likely pathogenic variant. Brain MRI scans for the proband (III-3) and other affected family members (III-4, III-8, and III-9) in their early twenties all illustrate variable degrees of prominent brain atrophy with ventriculomegaly (arrows) on axial T2-weighted images (left and middle panels) and cerebellar atrophy (arrow heads) on sagittal T1-weighted images (right panel).
We found a potential novel intragenic deletion in \textit{OPHN1} in a family with X-linked intellectual delay with cerebellar ataxia and dystonia. Missense variants and deletions in \textit{OPHN1} have been previously reported.\textsuperscript{52,53} Oligophrenin, encoded by \textit{OPHN1}, interacts with the Rho GTPase and is highly expressed in neurons, especially the hippocampus, basal ganglia, and cerebral cortex. Decreased oligophrenin leads to increased Rho activity and results in a perturbed neuronal development, which could explain the phenotypes and structural brain abnormalities seen on MRI scans. Notably, dystonia has not previously been reported in affected male subjects with \textit{OPHN1} substitutions. Our observations extend the clinical spectrum related to this syndrome. The co-occurrence of dystonia and neurodevelopmental delay has long been recognized as implicating causative genetic variants related to neurodevelopment, with a pathogenic substitution frequency of up to 45\% in complex dystonia syndrome.\textsuperscript{41,54} Our findings support the concept that genomic sequencing has allowed for characterization of new gene-to-disease relationships in patients with rare neurologic syndromes.

The current study has several limitations. First, we did not perform neuropsychological tests or peripheral nerve conduction studies in all enrolled patients, limiting subgroups to a category of patients with combined nonmotor features. A multicenter study using a clinical scoring algorithm that includes complex dystonia syndrome is needed. Second, the major genetic tool we applied in this study is targeted panel sequencing, which can miss variations in genes that have yet to be discovered or repeat expansions and mitochondrial genes that were not specifically targeted in this study. Furthermore, the very recently identified dystonia-linked genes, such as \textit{VPS16} and \textit{VPS4I}, were not included in this panel.\textsuperscript{55} Future studies using whole-exome or genomic sequencing in those patients without known causative genetic causes are needed to uncover novel dystonia-associated genes in our population. Third, although we identified several potential novel variants co-segregating with the phenotypes within the families, such as the \textit{C19orf12} p.P92Tfs*9 variant and the intragenic intron 1 deletion in \textit{OPHN1}, future functional experiments are needed to confirm the pathogenicity of these novel substitutions.

In conclusion, we present a systemic genetic analysis in a large Taiwanese cohort to elucidate the genetic substitution spectrum in patients with isolated or combined dystonia. A targeted panel is suitable for those with isolated dystonia or dystonia with myoclonus or parkinsonism in our population. However, if patients present with a more widespread or diverse clinical phenotype, especially those combined with non-neurologic development disorders, whole-exome or genomic sequencing will be suggested. Our results have the potential to facilitate accurate molecular diagnosis and careful clinical characterization to allow for more tailored genetic testing in patients with dystonia.
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Author Contributions


Supplemental Data

Supplemental material for this article can be found at http://doi.org/10.1016/j.jmoldx.2021.12.003.

References


**Supplemental Figure S1**  Pedigrees of patients with combined dystonia and myoclonus who were heterozygous for \textit{SGCE} pathogenic or likely pathogenic variants identified in the current study. Affected family members are represented with black circles (female) or squares (male). The number in the diamond indicates total number of children with unknown sex. Arrows indicate probands.

**Supplemental Figure S2**  Pedigrees of patients with combined dystonia and parkinsonism who were heterozygous for \textit{GCH1} (A) \textit{TH} or (B) pathogenic or likely pathogenic variants identified in the current study. Affected family members are represented with black circles (female) or squares (male). The number in the diamond indicates total number of children with unknown sex. Arrows indicate the proband.