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Monogenic variants in dystonia: an exome-wide sequencing study

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Summary

Background—Dystonia is a clinically and genetically heterogeneous condition that occurs in isolation (isolated dystonia), in combination with other movement disorders (combined dystonia), or in the context of multisymptomatic phenotypes (isolated or combined dystonia with other neurological involvement). Although our understanding of the underlying etiologies is incomplete, no large-scale genomic investigation has been performed. We aimed to elucidate the landscape of monogenic causes for the major clinical categories of dystonia.

Methods—We sequenced the exomes of 764 individuals with dystonia and 346 healthy parents, recruited between June-1, 2015, and July-31, 2019, from 33 specialty clinical and research centers located in Europe. Affected subjects assessed in this exome-based genetic testing study presented various types of isolated and combined dystonia (with or without coexisting symptoms) including manifestations clinically diagnosed as dystonic cerebral palsy (76/764). Using stringent sequence-data filtering and interpretation protocols, we performed an exome-wide search for causative variants in described disease genes. In the cases that went undiagnosed, candidate dystonia-causing genes were prioritized.

Findings—We identified causative or likely causative variants in 135 (19%) of 728 families, involving 78 distinct monogenic disorders. We observed a significantly higher overall presumptive

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Contributors

M.Z. and J.W. designed and oversaw the study. M.Z, J.W., R.J., S.B., S.W., C.Z., and K.O. performed primary interpretation of the data. M.Z. and J.W. wrote the manuscript. K.O. had major contributions to manuscript editing. R.J., S.B., M.S., A.J., J.N., K.V., F.D., M.S., M.K., A.F., P.H., J.R., I.P., M.A., O.U., K.B., P.D., B.V., V.H., P.P., Z.G., T.M., T.M., A.S, S.S., A.B., T.R., M.V.B., E.H., B.P., B.L., S.B., T.H., B.L., J.S., M.M., G.A.R.-R., K.Õ., L.P., I.M., V.P., S.P., J.V., M.T., F.F., F.W., M.E., M.H., M.B., M.W., D.W., T.M., J.V., A.S., K.C., T.S, F.Z., V.K., C.M., P.G-A., R.G., E.M., I.B., A.K., A.H., R.S., B.A., C.S., T.O., E.R., R.D.C., D.D., W.K.C., A.C.-B., B.H., and V.M. recruited and phenotyped participants. I.B. contributed to case discussions. V.M. contributed to the classification of participants with cerebral palsy and the concept of cerebral palsy. C.Z. performed in-silico modeling. S.G. and T.B.-B. performed and O.D. supervised functional studies. M.W., Y.D., M.K., M.H.W., S.P., K.Õ., U.A.S., F.L., R.C., A.I., H.P., E.G., D.S.W., K.M.R., T.B., B.A., R.B., T.M.S., J.B., T.M.B., L.J.O., A.V., S.B.W., and H.E. did or supervised genetic studies (whole-exome sequencing and/or sequence data analysis). R.P. supervised whole-exome sequencing and performed final sequence data analysis. A.T., R.E.P., F.M.Z., and L.B.H. did or supervised genetic studies at GeneDx. All authors contributed to and critically reviewed the final version of the manuscript.

Declaration of interests

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diagnostic rate for dystonia (either isolated or combined) with coexisting non-movement disorder-related neurological symptoms (100/222=45%, excepting cases with evidence of perinatal brain injury) than for combined (19/98=19%) and isolated (16/388=4%) dystonia. Across all categories of dystonia, 65% of the detected variants affected genes which are associated with neurodevelopmental disorders. We report 11 disease genes not previously linked to dystonia, and set forth a predictive clinical score that could guide the implementation of exome sequencing in routine diagnostics. Further, we demonstrate that in cases without perinatal sentinel events genomic alterations contribute substantively to the diagnosis of dystonic cerebral palsy. In 15 families, we delineated 12 candidate genes. These include *IMPDH2*, encoding a key purine biosynthetic enzyme, for which we provide strong evidence for involvement in a neurodevelopmental disorder with dystonia. We describe six individuals from four cohorts with spatially clustering *IMPDH2 de novo* variants, expected to result in deregulation of purine metabolism.

Interpretation—Our study determines the role of monogenic variants across the spectrum of dystonic disorders, providing guidance for the introduction of personalized care strategies and fostering follow-up pathophysiological explorations.

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Introduction

Defined by the presence of involuntary muscle contractions and abnormal postures, dystonia comprises a broad class of movement disorders[1]. Dystonia is a descriptive term rather than a specific diagnosis and characterized by vast heterogeneity with respect to phenomenology, comorbidity, and pathogenic mechanisms[1]. Dystonia is categorized as isolated if manifesting as the sole movement-disorder symptom, and combined if present in conjunction with other movement disorders[2]. In addition, dystonia can coexist with a variety of non-movement disorder-related neurological manifestations[2,3]. Although over 250 genes have been linked to the causation of dystonia[4,5], most molecular screening efforts have been based on single-gene and gene-panel analysis techniques. Three genomic sequencing studies in small, mostly preselected cohorts of dystonia-affected subjects have been published, reporting a restricted repertoire of variants in a minority of individuals investigated[4,6,7]. The contribution of the different implicated genes to dystonia remains poorly defined and the associated variant signatures have not been thoroughly elucidated. Moreover, it is likely that many individuals with dystonia carry pathogenic variation in as-yet-unidentified disease-relevant genes.

To enhance our knowledge about the genetics of dystonia, we generated whole-exome sequencing (WES) data for 1,110 individuals from 728 families. Using a two-stage analytic approach, we (i) performed an exome-wide search for known molecular etiologies and (ii) pursued the identification of candidate disorder-related genes by prioritizing high-impact variants on a case-by-case basis.

Methods

Study design and participants

For this exome-wide sequencing study, we recruited an unselected set of 764 affected individuals (728 index cases) and 346 healthy parents. The study participants were identified at 33 movement-disorder and neuropediatric specialty centers across Europe (Austria, Czech-Republic, France, Germany, Poland, Slovakia, and Switzerland). Each affected individual was diagnosed with dystonia in accordance with the dystonia consensus definition[2]. Index cases were eligible for this study if they had (1) no prior genetic diagnosis and (2) no indication of an acquired cause of their illness. We did not apply the latter criterion to a subset of participants with a working clinical diagnosis of dystonic cerebral palsy (DCP), because cerebral palsy has been shown to be subject to frequent misclassification[8] and we sought to systematically assess whether genetic factors contribute to the etiology of these individuals` conditions. To aid in the identification of candidate genes, we interrogated existing WES data of ~500 research participants who had been referred for a variety of neurodevelopmental disabilities including movement disorder. A further six case subjects presented in this work were obtained through GeneMatcher[9].

All participants or their guardians completed written informed consent, and the study protocol had been approved by the respective ethical committees.

Procedures

WES raw data of dystonia-affected individuals and healthy parents were produced at Helmholtz-Center-Munich (1089 samples) and two collaborating institutions (German-Cancer-Research-Center and Warsaw-Medical-University). Experimental procedures were performed according to methods detailed elsewhere[4]. Briefly, genomic DNA was extracted from blood and subjected to library construction using Illumina 100-bp paired-end protocols. Exons and proximal splicing elements were enriched with the Agilent (Agilent-Technologies, Santa Clara, CA; USA) SureSelect-Human-All-Exon-v5 (27% of samples) or -v6 (73% of samples) kit and sequenced on a HiSeq2500 or HiSeq4000 (Illumina, San Diego, CA, USA). For variant calling and annotation, reads were loaded into an in-house-developed pipeline (Helmholtz-Center-Munich and Technical-University-of-Munich) that incorporates a battery of publicly available bioinformatics tools and customized software (appendix-p-58)[4]. WES experiments on six trios found through GeneMatcher were conducted at GeneDx, Meyer-Children’s-Hospital, Massachusetts-General-Hospital, and Tartu-University-Hospital using previously reported sequencing methodologies (appendix-p-58).

Determination of (likely) causative alleles in described disorder-related genes followed a stringent methodology (appendix-pp-6-7). First, leveraging data from multiple sources[5,10–15], all exome-generated variants were filtered by occurrence in predefined gene-sets (appendix-pp-6-7; pp-35-36), predicted effect on protein, disease inheritance, minor allele frequency (MAF), and known or expected pathological impact. Filtered variants were subjected to co-segregation analyses and unreported variants were classified according to the American-College-of-Medical-Genetics-and-Genomics guidelines[16]. Second, all considered

variants were reviewed in expert roundtable sessions to validate their clinical significance. Variants that survived our filtering and interpretation procedures were defined as diagnostic variants.

To prioritize candidate dystonia-related genes, a stepwise workflow was implemented (appendix-pp-8-9). Variants were assessed under different inheritance models and filtered by MAF and predicted effect on protein function. We gave priority to variants in brain-expressed genes and considered various metrics of variant severity including computational pathogenicity predictions[13–15] and gnomAD scores of genetic constraint[10]. We scrutinized in-house WES data to identify additional individuals with likely deleterious variants in the gene of interest and sought supportive evidence from the literature. All genes nominated through our approach were submitted to GeneMatcher[9].

All diagnostic and candidate variants were confirmed with an independent method (appendix-p-58).

Statistical analysis

Statistical analyses were carried out using R (version 3.2.3, “stats-package”). We assessed the significance of association between an individual having a diagnostic WES finding and clinical characteristics by using Pearson chi-square tests. To identify factors strongly predictive of a WES-based diagnosis, we undertook multivariable logistic regression analysis. The effect of significant predictor variables was used to devise a scoring system quantifying the probability of reaching a diagnostic conclusion through the application of WES. Details on the score construction are described in the appendix (p-3).

Three-dimensional modeling of candidate variants and biological system analyses were conducted using standard computational methods (appendix-p-59). To study the temperature-dependent unfolding behavior of wild-type and variant-bearing forms of the candidate IMPDH2, we used an *E.coli*-expression system and performed thermal-shift assays (appendix-p-59).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 1,110 participants recruited between Jun-1, 2015, to Jul-31, 2019, 526 were analyzed as singletons, 58 as duos, 498 as parent-offspring trios, and 28 as quartets. In accordance with our inclusion criteria, 76 index cases were diagnosed with DCP. The DCP-affected subjects were subdivided into two groups[17], idiopathic DCP (56 cases) and non-idiopathic DCP (20 cases) (appendix-p-10). Individuals from the latter group, who had evidence of perinatal brain injury by either history and/or brain MRI, underwent genomic exploration as part of the present study, but were excluded from the statistics described below. A total of 708 index cases had dystonia of unknown etiology and are hereafter collectively referred to

as the “dystonia cohort” (Tab. 1; appendix-pp-11-12). Details of the subgrouping and clinical characteristics of the cohort are provided in Tab. 1 and the appendix (p-11). Sequencing metrics are summarized in the appendix (p-13).

We established 78 distinct presumptive molecular diagnoses in 135 index cases (19.1%) of the dystonia cohort (108 newly reported, 27 described previously); Fig. 1-2 and data in the appendix (pp-14-20; pp-37-47) summarize the molecular-genetic findings and the phenotypes involved. When analyzed by dystonia category, diagnostic variants were detected in 4.1% (16/388) of cases with isolated dystonia, 19.4% (19/98) of cases with combined dystonia, and 45.0% (100/222) of cases with dystonia (either isolated or combined) and coexisting non-movement disorder-related neurological symptoms (35.2% for isolated dystonia with coexisting symptoms; 49.7% for combined dystonia with coexisting symptoms; Fig. 1; Fig. 2A). The majority of presumptive diagnoses (71.8%) were observed only once in this study. The most commonly implicated gene was *KMT2B* (8.9% of the diagnoses), followed by *SGCE* (4.4%) (appendix-p-14). Forty (51.9%) of 77 involved genes were associated with autosomal-dominant disorders, 32 (41.6%) with autosomal-recessive disorders, 4 (5.2%) with X-linked disorders, and 1 with both an autosomal-dominant and an autosomal-recessive disorder (appendix-pp-37-43). Of the 88 total variants related to autosomal-dominant and X-linked disorders, 52 (59.1%) were confirmed to be *de novo* (Fig. 2B; appendix-pp-37-43). We identified a full range of variant types (Fig. 2C), with missense changes representing the major class of diagnostic variants (59.4%). More than half of the variants (91/160=56.9%) were novel at the time of our analysis (Fig. 2D; appendix-pp-37-47). Eighty-six (94.5%) of these novel alterations fulfilled criteria of being pathogenic or likely pathogenic[16]; the remaining 5 were formally classified as variants of uncertain significance (VUS)[16] but considered diagnostic based on phenotypic overlap between respective carriers and earlier described cases (appendix-pp-44-47).

Of the 135 index cases with a presumptive diagnosis, 94 (69.6%) harbored variants in genes previously associated with neurodevelopmental disorders (NDDs) (appendix-pp-48-49)[5,18]. The highest contribution of variants in NDD-associated genes was found among cases with dystonia and coexisting non-movement disorder-related neurological symptoms (83.0%; appendix-p-18). Consistent with this, this subgroup displayed an array of developmental disabilities and accompanying features (appendix-p-19). In isolated and combined dystonia (without additional symptoms), 31.3% and 31.6% of the diagnosed case presentations were attributable to variants in neurodevelopmental genes respectively. For 11 (20.4%) of the 54 NDD-associated genes, a link to dystonia has not been described before (Fig. 2E); these included genes for which only a handful of families with pathogenic variants have so far been reported (*DHCR24*, *GRID2*, *MORC2*, *MSL3*, *PAK1*, *PPP2R5D*, *TECPR2*, and *ZMYND11*), but also genes whose associated trait manifestations have been more extensively characterized (*AUTS2*, *CHD8*, and *ZEB2*). Twelve of the 13 cases with variants in these genes had combined dystonia, and all had non-movement disorder-related comorbidities (appendix-p-19).

Forty-six index cases, accounting for 6.5% of the cohort, had diagnostic findings with potential impact on clinical management (Fig. 2F; appendix-pp-50-51). Thirty-two presumptive diagnoses provided grounds for the administration of available therapies

(medical diets, cofactor-supplementation, specific medications), and another 14 led to initiation of individualized surveillance measures. To examine whether the likelihood of receiving a WES-based diagnosis was related to the presence of certain clinical variables, we performed within-cohort comparisons of diagnostic yield. Chi-square tests showed significant differences in the yields between subgroups separated by age at dystonia onset, distribution of dystonia, dystonia category, current age, and occurrence of brain-MRI abnormalities (all $p<0.0001$; appendix-p-21). No significant difference in the diagnostic rates was seen between subgroups separated by leading motor phenomenology. In a multivariable logistic regression, independent predictors for a WES-based diagnosis were determined as follows: onset of dystonia before the age of 21 years; a segmental or generalized distribution of dystonia; and a presentation of combined dystonia or dystonia (isolated or combined) with coexisting non-movement disorder-related neurological symptoms (all $p\leq0.005$; appendix-pp-52-53). We developed a 7-component scoring system that allows clinicians to identify dystonia-affected individuals who are most likely to benefit from WES (appendix-p-3; pp-52-53; Fig. 3). We assigned scoring points to each predictor variable according to the magnitude of the regression coefficients (appendix-pp-52-53) and obtained summary scores ranging from 0–5 points (Fig. 3A-B; appendix-p-22). We defined summary scores of 3 and 5 points as cutoff levels for clinical decision-making (Fig. 3B). By applying a cutoff score of 3, 130 cases (96.3%) with a presumptive diagnostic finding were correctly identified; the associated sensitivity was 96.3%, and the negative predictive value was 98.6%. The proposed algorithm for incorporation of WES in dystonia diagnostics is presented in Fig. 3C.

We present in this work molecular-genetic findings in an unselected sample of 76 DCP-affected individuals, of whom 56 had no apparent perinatal brain damage (appendix-p-10); 20 cases with non-idiopathic disease were investigated to test for the presence of genetic lesions that may have increased vulnerability to environmental factors[17]. We detected diagnostic variants in 37 cases of the idiopathic DCP group (66.1%), whereas no molecular diagnoses were achieved for cases with non-idiopathic DCP (Fig. 4A). The observed variants were associated with 27 different disease entities, of which 24 (88.9%) have previously been characterized as NDDs (Fig. 4B; appendix-pp-48-49)[5,18]. *SPAST* was the most frequently implicated gene, with *de novo* missense variants identified in 5 cases (appendix-p-23). Originally described as causative of a late-onset pure form of spastic paraplegia, *SPAST* has been recently shown to be involved in childhood complex presentations[19]. Our cases displayed a spectrum of developmental abnormalities (appendix-p-19); generalized dystonia was seen in 4 cases, and focal dystonia in one. These results add to the delineation of a pediatric syndrome caused by *SPAST* variants and indicate that this condition may underlie a small but considerable number of DCP diagnoses.

Having characterized the contribution of variants in known disease genes to dystonia, we sought to expand the molecular etiology underlying the disease. In a case with infancy-onset dystonia and other neurological manifestations, we discovered a *de novo* missense variant (p.Gly113Glu) in *IMPDH2*, predicted to disrupt an invariant residue within the cystathionine-β-synthase (CBS) domain of the encoded protein (Fig. 5A; appendix-pp-24-26). *IMPDH2* encodes inosine-5`-monophosphate dehydrogenase 2, a key enzyme in the purine biosynthetic pathway, expressed throughout the brain[20] and not linked previously

to any human Mendelian condition. A variety of genetic syndromes have been shown to be mediated by defects in CBS domains, including retinal dystrophies caused by missense variants in *IMPDH1*[21]. A further *de novo* substitution mapping to codon-113 (p.Gly113Arg) was found in in-house WES data from NDD-affected individuals. Moreover, through GeneMatcher[9], we identified the missense variants p.Gln243His and p.Gly207Arg (2x) and the single amino-acid deletion p.Ser160del, each of which had arisen *de novo*. The 6 variants were predicted to be deleterious and none of them were seen in control databases (appendix-p-54). All affected conserved amino-acids and resided in and around the CBS domain (Fig. 5B; appendix-p-24). The variant carriers shared similar neurodevelopmental phenotypes (appendix-pp-3-5; p-24). Apart from the dystonia-cohort index case, only 1 subject had evidence of dystonic posturing. Modeling of the variants on 3D protein-structures revealed spatial clustering near specific functional sites, predicted to result in deregulation of IMPDH2 activity (appendix-pp-24-26)[22]. In addition, thermal-shift assays demonstrated that variants identified within (p.Gly207Arg) and in close vicinity to (p.Gln243His) the CBS domain affected the stability and/or folding behavior of IMPDH2 (appendix-pp-25-26). Together, our genetic, clinical, and molecular-characterization data, supported by the fact that variants in other purine biosynthetic genes are well-recognized causes of NDDs with phenotypes similar to those observed here (appendix-p-24)[5] provided strong evidence for the pathogenicity of the *IMPDH2* variants.

We prioritized 11 additional candidate genes, 7 affected by dominant-heterozygous variants (*BAZ1B*, *CHD6*, *KIAA1244*, *KLC1*, *SPTBN1*, *ZNF532*, and *ZNF629*) and 4 by autosomal-recessive variants (*AOPEP, CDKL1*, *GIGYF1*, and *LINGO4*) (appendix-pp-27-29; pp-54-57). Four of these candidates (*CHD6*, *KIAA1244*, *LINGO4,* and *ZNF532*) were found to harbor variants in 2 or more independent families; the remaining 7 genes were each identified in only a single index case. None of these 11 genes have been associated with a monogenic disorder before. Phenotype information and evidence supporting candidacy of the genes are described in detail in the appendix (pp-54-57).

To elucidate biological processes perturbed by dystonia-associated variation (affecting known and candidate disease genes), we conducted gene-set enrichment analyses (appendix-p-59). There was significant enrichment of genes involved in dopaminergic signaling, nucleotide-binding, and nervous-system development (appendix-pp-30-31). We also evaluated to what extent the input genes were associated with phenotypes other than dystonia and found that a diverse array of HPO-terms were overrepresented (appendix-pp-32-33). Lastly, we generated a functional relatedness network to identify 20 genes that may serve as additional candidates for involvement in dystonia (appendix-p-34).

Discussion

By applying exome-wide testing to a diverse collection of dystonia-affected families, we were able to obtain a presumptive diagnosis in 19.1% of index cases (135/708, excepting those with evidence of perinatal brain damage). Another 2.1% of cases had candidate gene findings.

Our assessment of known and putative novel monogenic etiologies allowed us to make several key observations.

First, we found significant differences in the relative contributions of (likely) causative variants to distinct categories of dystonia. We attained the greatest variant-detection rate (45.0%) in individuals with dystonia (either isolated or combined) and coexisting non-movement disorder-related neurological symptoms, followed by a rate of 19.4% in individuals with combined dystonia. The high overall presumptive diagnostic yield in dystonia with coexisting symptoms was largely driven by variation in genes known to be linked to NDDs. Although the co-occurrence of dystonia and NDDs has long been acknowledged[23], there have been no previous studies specifically investigating the genetic basis of this relationship. Our finding that 69.6% of the presumptive diagnoses were related to NDD-associated genes implies that dystonia and NDDs exhibit substantial etiologic overlap on the genomic level. This is in line with data from animal models, having stimulated the translation of basic neurodevelopmental concepts into the area of clinical research in dystonia[24,25]. Notably, we identified diagnostic alterations in 11 genes associated with neurodevelopmental conditions that were not previously known to include dystonia as a phenotype (NDDs related to *AUTS2*, *CHD8*, *DHCR24*, *GRID2*, *MORC2*, *MSL3*, *PAK1*, *PPP2R5D*, *TECPR2*, *ZEB2*, and *ZMYND11*). We suggest that recognizing dystonia as a presenting feature of a complex neurological illness should raise suspicion of an underlying NDD and prompt a thorough search for variants in NDD-associated genes, including those described in this study. The reasons why 95.9% of cases with isolated dystonia remained without a genetic diagnosis may be heterogeneous. We assume that (i) a number of monogenic causes in isolated dystonia are yet to be discovered, and (ii) polygenic variation of individually low effect size and/or environmental influences may constitute major etiologic factors for this type of disease.

Building on existing clinico-genetic paradigms[1], we demonstrated that specific phenotypic aspects can predict the diagnostic success rate of WES. Individuals who were diagnosed had significantly earlier age-at-onset of dystonia, significantly more widespread dystonia, and significantly more often additional neurological symptoms. It is often unclear which subjects should be evaluated with WES[26], especially in the field of dystonia. We designed a weighted clinical score to optimize the utilization of WES-based testing. Following prospective validation, we hope that the score will be incorporated in workflows for etiologic evaluation of dystonia[27], thereby leading to earlier accurate diagnoses. This in turn would have important implications for precision-medicine strategies, as reflected in our data showing that 34.1% of the presumptive diagnoses had the potential to shape medical management. A limitation of our scoring algorithm is the size of input data for cases with isolated dystonia which does not achieve adequate power to infer individual testing recommendations in this subgroup. Adaption of the scoring items may be necessary when more data on genomic variation in additional dystonia cohorts become available. The fact that we did not incorporate internal validation when developing the algorithm represents an additional limitation, potentially resulting in too optimistic estimates of its predictive ability. Another pertinent question in exome diagnostics is how variants are being evaluated. We acknowledge that we cannot be 100% certain about the disease-causing nature of every single variant implied in the construction of the score, but we do not expect this limitation to

alter our key results. Although there is a chance of overestimating the overall burden of causative variants (5/160 variants formally qualified as VUS)[16], the number of false positives is likely to be small given that all considered variants were subjected to rigorous filtering and multidisciplinary clinical interpretation protocols.

Second, our findings strongly support the conceptualization of cerebral palsy as a collection of neurodevelopmental Mendelian syndromes[28]. We detected diagnostic variants in two-thirds (66.1%) of subjects ascertained to have idiopathic DCP. Six genes were recurrently impacted by (likely) causative alleles in DCP. One important example involves *SPAST* for which anecdotal reports have described a connection to DCP-like entities[19,29]. We stress the need for accurate (re-)examination of individuals diagnosed with *SPAST*-related disease to determine the relative phenotypic contributions of dystonia and spasticity, 2 features not readily distinguishable in the context of multisymptomatic motor disorders. Having elucidated the crucial role of monogenic variants in the causation of DCP, our study may serve as a starting point for the establishment of optimized care pathways and targeted clinical trials in this heterogeneous group of disorders. Notably, no genomic predisposing disorders were found among 20 cases with non-idiopathic DCP. This leads us to emphasize the importance of careful clinical evaluation before integrating WES in the etiologic workup of DCP.

Finally, our approach enabled the prioritization of 12 candidate genes. Although additional research is required to establish their *bona-fide* link to disease, our study provides evidence supporting plausible candidacy. We give the highest level of confidence to *IMPDH2*, in which heterozygous variants were identified in 6 unrelated cases. We note that the phenotype in cases with *IMPDH2* variants was primarily one of a NDD, and that dystonia was variably expressed (2/6). This clinical outcome is in good agreement with that produced by disruptions of other genes involved in purine metabolism such as *HPRT1*, variants of which cause Lesch-Nyhan syndrome, a NDD with varying degrees of dystonia[30]. We also emphasize the putative candidacy of the remaining 11 genes that we propose on the basis of expected high-impact variants and other lines of evidence. Moreover, we performed enrichment and interactome studies, revealing converging processes and additional genes potentially related to the evolution of dystonia.

Collectively, we have created a comprehensive view of causal and likely causal genetic variation across the entire range of dystonic disorders. We highlight diagnostic overlap between dystonia and NDDs, propose a framework for integrating WES into routine diagnostics, and demonstrate the genetic burden of disease in individuals with DCP. Conclusions based on our data suggest that genomic testing should be considered a first-visit diagnostic strategy in individuals (especially those with early disease-onset) who have (i) isolated dystonia with additional neurological involvement, (ii) combined dystonia (with or without other neurological symptoms), and (iii) a diagnosis of DCP that is not explainable by perinatal brain-injury. Moreover, our study adds evidence that defects in purine metabolism contribute to dystonia and represents the basis for future discoveries of dystonia-related genes.

Supplementary Material

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Research in context

Evidence before this study

We searched PubMed with the terms “dystonia AND exome sequencing” and “dystonia AND genome sequencing” for manuscripts published before March 01, 2020, without restrictions on language of publication. Two studies had used whole-exome sequencing and one study had used whole-genome sequencing in small collections of dystonia-affected probands. Our 2017 pilot project applying whole-exome sequencing detected disease-causing variants in 6 of 16 cases (37.5%) with early-onset generalized dystonia. The other study employing whole-exome sequencing reported a diagnostic yield of 20.2% in 189 probands with dystonia. The third study, describing the results of whole-genome sequencing in a cohort of mostly isolated dystonia-affected probands (111 families), established a molecular diagnosis in 11.7% of cases. No study elaborated the role of monogenic disorders in the etiology of dystonia in a comprehensive manner, provided a framework for translating findings into genetic testing recommendations, or integrated the prioritization of novel disease-causing genes.

Added value of this study

We performed an exome-wide sequencing study on 1,110 individuals from 728 families to illuminate the molecular landscape of dystonia. First, we demonstrate the highest contribution of monogenic variants to disease etiology for cases in which dystonia was combined with additional neurological features (other movement disorders and/or non-movement disorder-related symptoms). In this subgroup, genetic conditions previously characterized as neurodevelopmental disorders accounted for the majority of presumptive diagnoses, strengthening reported links between brain developmental processes and the pathophysiology of dystonia. We also uncovered likely causative variants in known disease genes with no previous evidence of being related to dystonia, thus extending their associated phenotypic spectra. Uniquely powered by the scale of our data, we developed a scoring system that predicts the diagnostic power of exome sequencing and will help clinicians to prioritize dystonia-affected individuals who are most likely to benefit from exome-based testing. Second, we were able to reveal the strong monogenic basis of dystonic cerebral palsy. This finding challenges the long-held belief that these heterogeneous syndromes are mainly due to secondary causes (e.g., hypoxic brain injury) and allows for a more precise delineation of what is subsumed under the label “cerebral palsy”, assisting clinical management and prognostication. Finally, we identified *IMPDH2*, encoding a key enzyme of guanine nucleotide synthesis, as a putative novel dystonia-causing gene, expanding the catalogue of purine metabolism disorders, and we propose 11 other novel dystonia-causal gene candidates.

Implications of all the available evidence

By laying ground for the establishment of improved diagnostic algorithms and redefining etiologic thinking, our data are expected to have far-reaching implications for clinical care in the field of dystonia. Furthermore, the elucidation of the spectrum of known and

putative novel monogenic causes will enable a deeper understanding of the involved pathophysiological pathways and pave the way for future research initiatives.

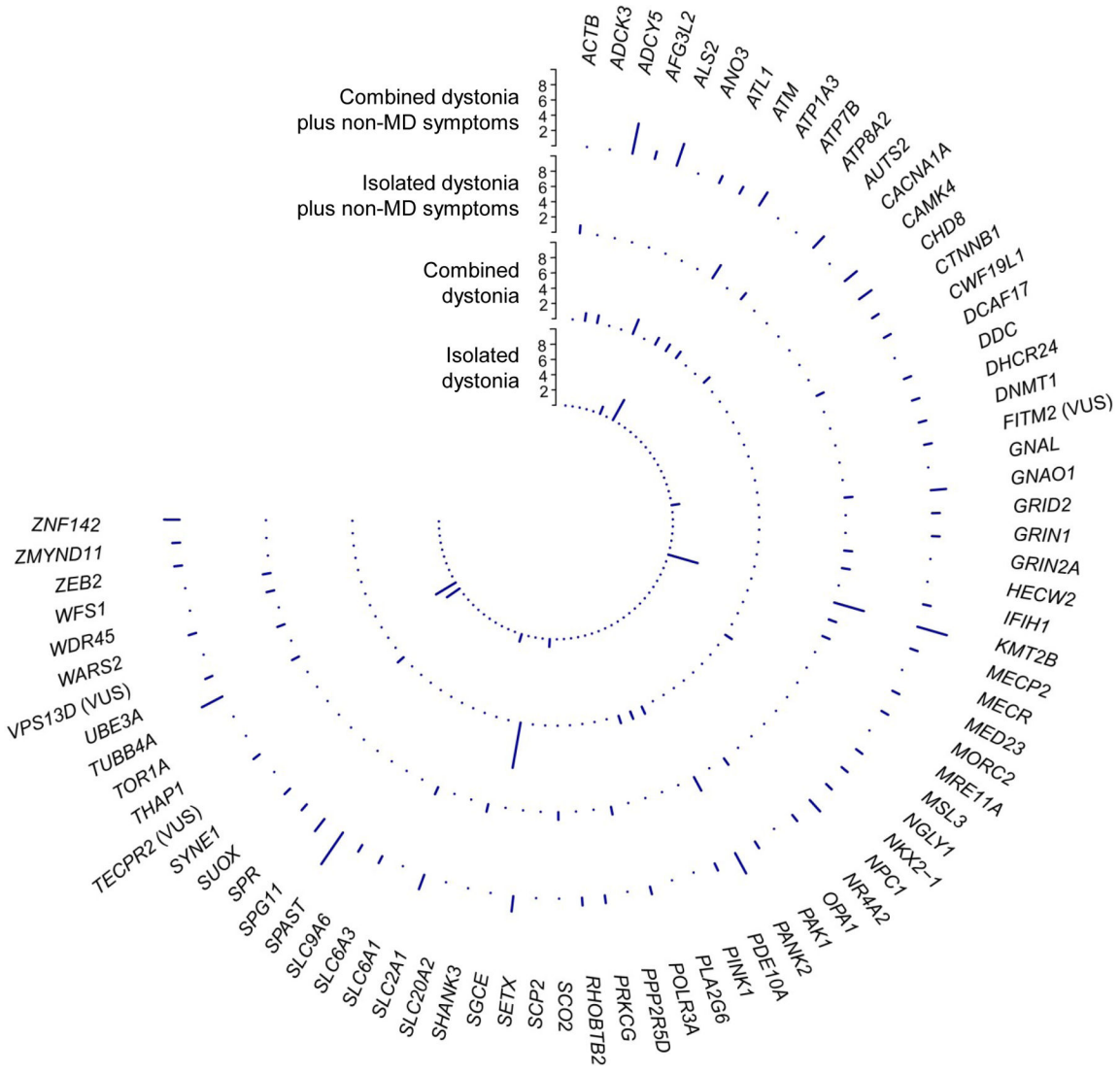

Figure 1. The landscape of genetic etiologies in the dystonia cohort

(A) Spectrum of genes containing diagnostic variants. By using WES, a total of 135 presumptive diagnoses were established, representing 78 distinct disease entities. The counts of individuals with presumptive diagnosis per gene are indicated for different dystonia clinical categories. VUS refers to genes in which variants were formally classified as variants of uncertain significance[16]. Non-MD symptoms, non-movement disorder-related neurological symptoms.

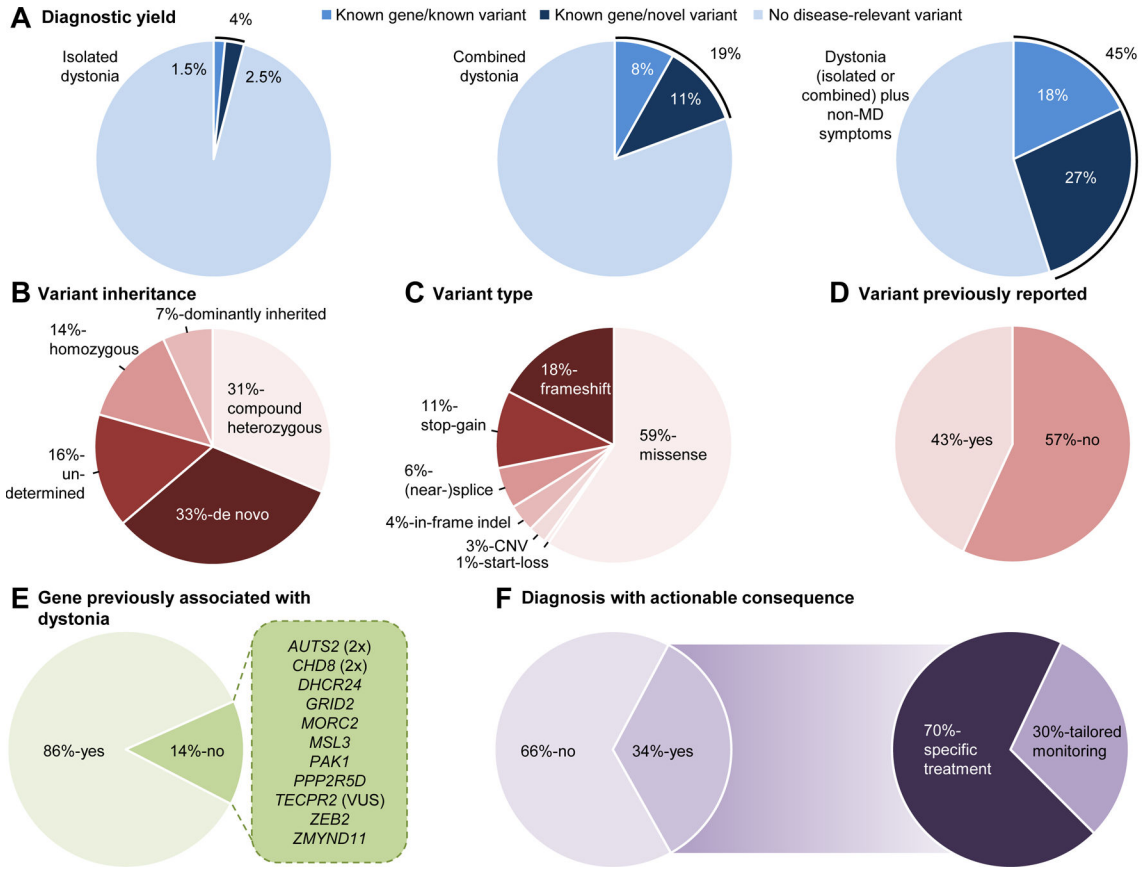

Figure 2. Pie charts summarizing the molecular diagnostic results for the dystonia cohort

(A) Overall presumptive diagnostic rates for different dystonia clinical categories, represented by 388 (isolated dystonia), 98 (combined dystonia), and 222 (isolated or combined dystonia with coexisting non-movement disorder-related neurological symptoms) individuals. “Known gene/novel variant” includes genes with compound heterozygous variants if at least one identified variant was novel. Non-MD symptoms, non-movement disorder-related neurological symptoms. (B) - (D) Characteristics of diagnostic variants. Distribution of variants by inheritance pattern (B) and type (C). Percentages shown for “variant inheritance” and “variant type” do not total 100 because of rounding. Of the 160 variants identified, 91 were not found in ClinVar or the literature (D). Additional variant characteristics can be found in the appendix (p-16) (E) Proposed novel associations between known disease genes and dystonia. There were 13 individuals carrying diagnostic variants in 11 genes (green box) whose dystonia manifestations were interpreted as expansions of the previously appreciated gene-specific phenotypes. For the remaining 66 variant-harboring genes, dystonia and/or (dystonic) tremor has already been documented to be part of the associated disease spectra. VUS refers to a gene in which variants were formally classified as variants of uncertain significance[16]. (F) Fraction of 135 WES-based diagnoses that pointed towards a clinical management and/or treatment implication. Breakdown by practical importance is shown (for details, see appendix pp-50-51).

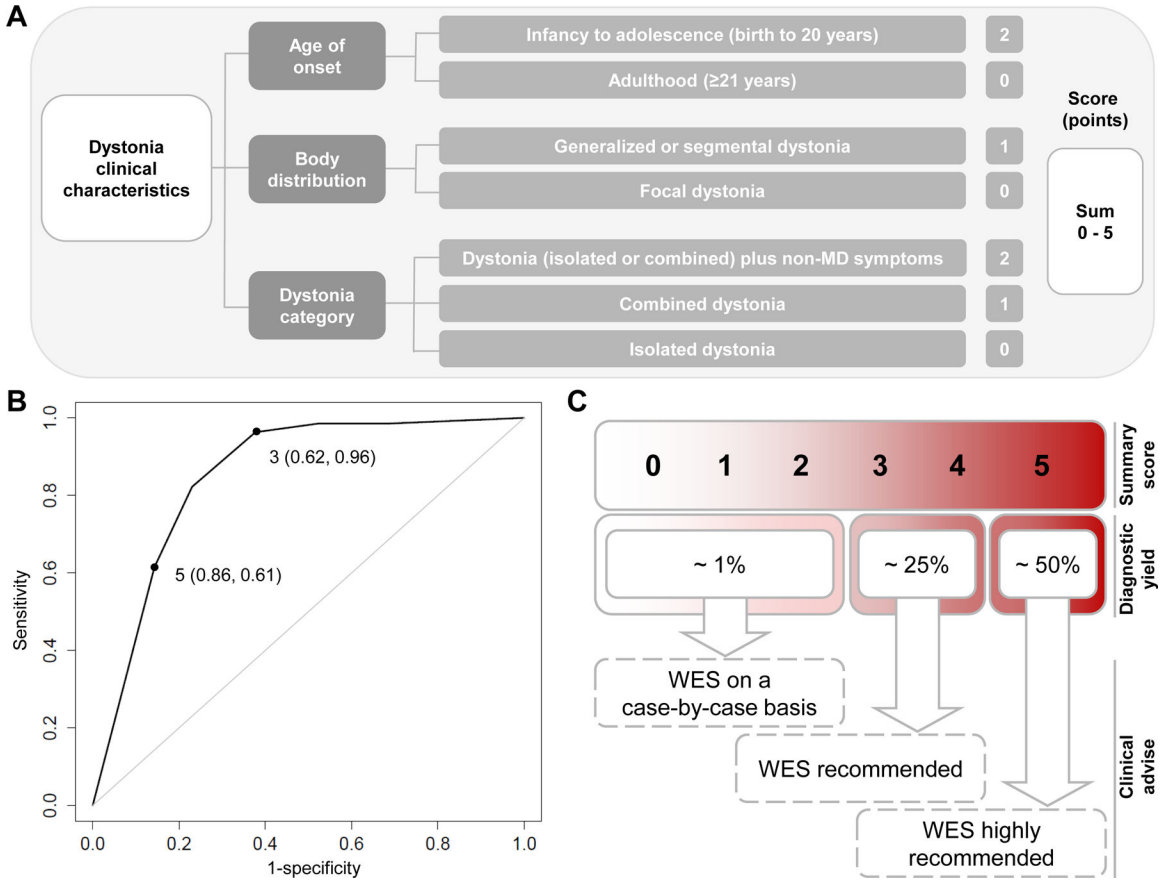

Figure 3. Clinical score predicting the diagnostic success rate of WES in individuals with dystonia

(A) Schematic overview of the proposed scoring system. We selected as scoring parameters clinical predictors of a diagnostic WES finding, as determined by multiple logistic regression analysis (appendix pp-52-53). The assigned scoring points add up to yield a summary score, ranging from 0–5. Non-MD symptoms, non-movement disorder-related neurological symptoms. (B) Receiver operating characteristic (ROC) curve plot for the proposed score with indication of the specificities and sensitivities (specificity, sensitivity) at the thresholds postulated in (C). A summary score threshold of 3 points implies a low rate (4%) of individuals that are erroneously excluded from WES and an acceptable rate (38%) of erroneously included subjects. (C) Summary scores (0–5), diagnostic yields, and recommendations for the clinical application of WES in dystonia. On the basis of our diagnostic WES results and multivariable logistic model, we suggest to routinely implement WES in dystonia diagnostics if the derived summary score is 3 or higher.

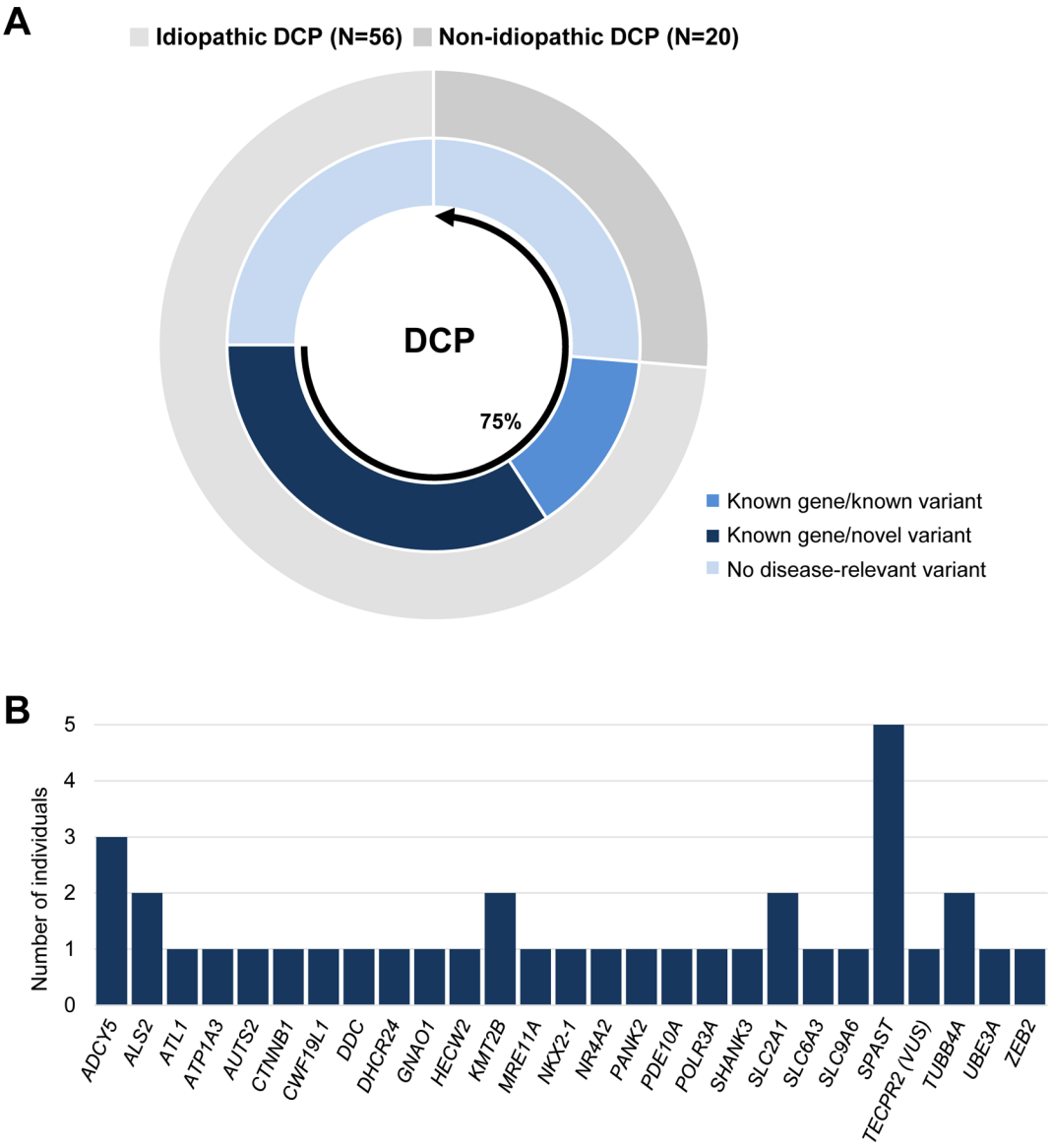

Figure 4. The landscape of genetic etiologies associated with dystonic cerebral palsy

(A) Double ring diagram correlating dystonic cerebral palsy (DCP) subtypes (outer ring) and the number of individuals who received a presumptive diagnosis via WES (inner ring). "Known gene/novel variant" includes genes with compound heterozygous variants if at least one identified variant was novel. Of the individuals with idiopathic DCP, 66% had a diagnostic variant. Overall, we were able to define the (likely) etiology of disease for three quarters of DCP-affected cases (variant-positive individuals plus individuals who showed evidence of perinatal brain injury). (B) Spectrum of genes containing diagnostic variants in the DCP cohort. By using WES, a total of 37 presumptive diagnoses were established, representing 27 distinct disease entities. The counts of variant-harboring individuals per gene are shown. *SPAST* carried diagnostic variants in 9% of individuals with idiopathic DCP. VUS refers to a gene in which variants were formally classified as variants of uncertain significance[16].

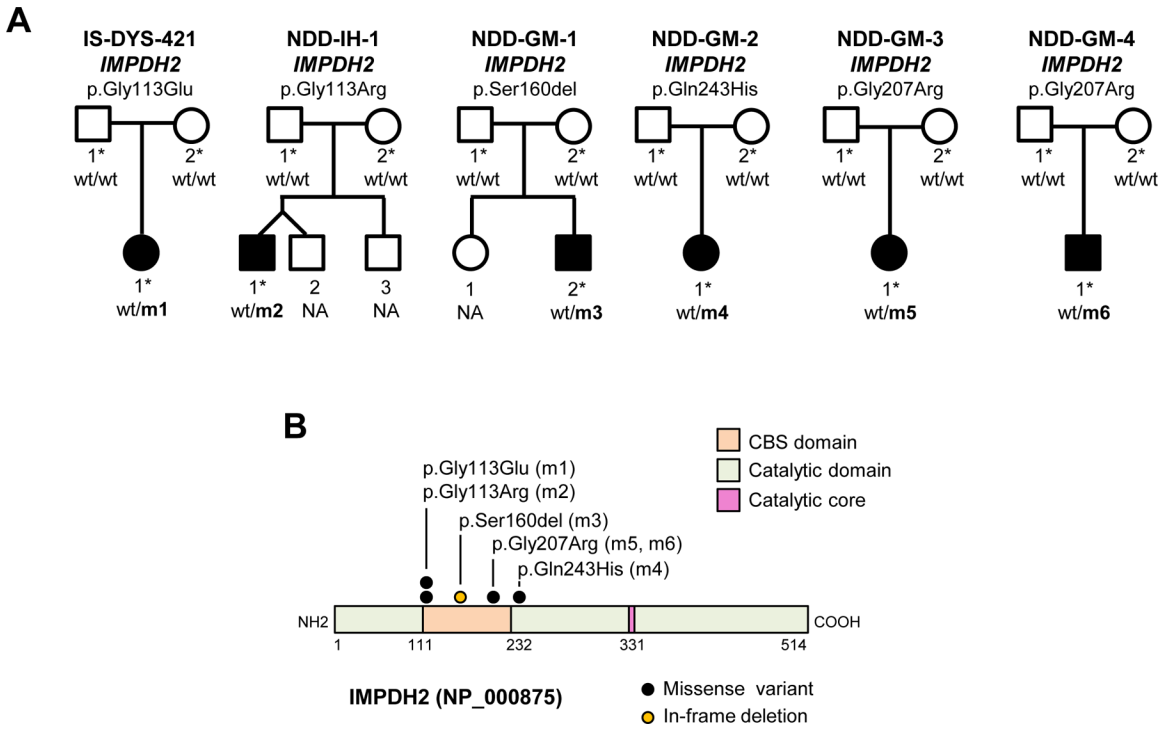

Figure 5. Spatially clustering *de novo* variants in *IMPDH2* are associated with a neurodevelopmental disorder with or without dystonia

(A) Pedigrees with *de novo IMPDH2* variants. Symbols are defined as follows: square, male; circle, female; filled, neurodevelopmental disorder (NDD)-affected (with or without dystonia); empty, unaffected; wt/wt denotes homozygous wild-type sequence, and wt/m1–6 denotes a heterozygous *IMPDH2* variant; m1, c.338G>A (p.Gly113Glu); m2, c.337G>A (p.Gly113Arg); m3, c.478_480delTCC (p.Ser160del); m4, c.729G>C (p.Gln243His); m5, c.619G>C (p.Gly207Arg); m6, c.619G>A (p.Gly207Arg); NA, no DNA available. All families were analyzed by trio WES (indicated with asterisks). (B) Linear view of inosine-5`-monophosphate dehydrogenase 2 (IMPDH2). The herein described variants are situated in and around the cystathionine-β-synthase (CBS) domain, a regulatory element in which clustering of pathogenic missense variants has been already demonstrated for IMPDH2`s homolog, IMPDH1[21,22]. Note variant recurrences at positions gly113 and gly207.

Table 1

Demographic, clinical, and sequencing characteristics of the dystonia cohort[a]

	Subcohort by dystonia clinical category[b]		Entire cohort N=708 (100)
	Isolated dystonia N=459 (64.8)	Combined dystonia N=249 (35.2)	
Demographics			
Gender			
Women	269 (58.6)	137 (55.0)	406 (57.3)
Men	190 (41.4)	112 (45.0)	302 (42.7)
Ancestry			
European	450 (98.0)	213 (85.5)	663 (93.6)
Middle Eastern	4 (0.9)	20 (8.0)	24 (3.4)
Turkish	3 (0.7)	6 (2.4)	9 (1.3)
Asian	2 (0.4)	6 (2.4)	8 (1.1)
South American	-	4 (1.6)	4 (0.6)
Age at testing			
Infancy to childhood (birth to 12 years)	44 (9.6)	72 (28.9)	116 (16.4)
Adolescence (13 to 20 years)	32 (7.0)	34 (13.7)	66 (9.3)
Adulthood (≥21 years)	383 (83.4)	143 (57.4)	526 (74.3)
Positive family history[c]	138 (30.1)	47 (18.9)	185 (26.1)
Dystonia clinical characeristics			
Age of onset			
Infancy to childhood (birth to 12 years)	115 (25.1)	163 (65.5)	278 (39.3)
Adolescence (13 to 20 years)	69 (15.0)	28 (11.2)	97 (13.7)
Adulthood (≥21 years)	275 (59.9)	58 (23.3)	333 (47.0)
Body distribution			
Generalized	112 (24.4)	137 (55.0)	249 (35.2)
Segmental	105 (22.9)	64 (25.7)	169 (23.9)
Focal	242 (52.7)	48 (19.3)	290 (41.0)
Diagnosed with idiopathic DCP[d]	7 (1.5)	49 (19.7)	56 (7.9)
Additional clinical characteristics			
Other movement disorder(s)			
Myoclonus	-	68 (27.3)	68 (9.6)
Parkinsonism	-	36 (14.5)	36 (5.1)
Choreiform movements	-	37 (14.9)	37 (5.2)
Ataxia	-	73 (29.3)	73 (10.3)
Spasticity	-	92 (36.9)	92 (13.0)
Non-movement disorder-related symptoms			
Developmental delay/hypotonia	58 (12.6)	125 (50.2)	183 (25.8)
Intellectual disability	43 (9.4)	75 (30.1)	118 (16.7)
Speech disorder	19 (4.1)	70 (28.1)	89 (12.6)

	Subcohort by dystonia clinical category[b]		Entire cohort N=708 (100)
	Isolated dystonia N=459 (64.8)	Combined dystonia N=249 (35.2)	
Seizures/epilepsy	22 (4.8)	40 (16.1)	62 (8.8)
Other neurological features	43 (9.4)	83 (33.3)	126 (17.8)
Leading motor phenomenology[e]			
Dystonia-predominant manifestation	56/71 (78.9)	158 (63.5)	214/320 (66.9)
Brain MRI abnormality[f]	50/348 (14.4)	106/219 (48.4)	156/567 (27.5)
Prior genetic testing (non-WES)			
Single-gene analysis	177 (38.6)	56 (22.5)	233 (32.9)
Gene-panel analysis	37 (8.1)	40 (16.1)	77 (10.9)
Chromosomal microarray analysis	31 (6.8)	62 (24.9)	93 (13.1)
Unknown	74 (16.1)	67 (26.9)	141 (19.9)
Sequencing mode (WES)[g]			
Solo	373 (81.3)	147 (59.0)	520 (73.4)
Duo	15 (3.3)	14 (5.6)	29 (4.1)
Trio	70 (15.3)	82 (32.9)	152 (21.5)
Quartet	1 (0.2)	6 (2.4)	7 (1.0)

[a]Number of individuals (percentage).

[b]According to Albanese et al.[1]

[c]Number (percentage) reported to have first/second degree relatives with dystonia and/or tremor and/or a multisymptomatic neurological phenotype related to the condition of the index case.

[d]Number (percentage) diagnosed clinically as having dystonic cerebral palsy (DCP) without evidence of perinatal brain injury.

[e]Refers to all individuals with combined dystonia and to individuals with isolated dystonia and coexisting non-movement disorder-related neurological symptoms.

[f]Brain magnetic resonance imaging (MRI) data available for 567 individuals of the cohort (80.1%).

[g]Solo, exome analysis of the index case; duo, exome analysis of the index case and 1 affected family member (affected parent or affected sibling); trio, exome analysis of the index case and the unaffected parents; quartet, exome analysis of the index case and the unaffected parents plus 1 affected sibling.

1. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord* 2013; 28: 863–73.