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Assessment of a Targeted Gene Panel for Identification of Genes Associated With Movement Disorders

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IMPORTANCE Movement disorders are characterized by a marked genotypic and phenotypic heterogeneity, complicating diagnostic work in clinical practice and molecular diagnosis.

OBJECTIVE To develop and evaluate a targeted sequencing approach using a customized panel of genes involved in movement disorders.

DESIGN, SETTING AND PARTICIPANTS We selected 127 genes associated with movement disorders to create a customized enrichment in solution capture array. Targeted high-coverage sequencing was applied to DNA samples taken from 378 eligible patients at 1 Luxembourgian, 1 Algerian, and 25 French tertiary movement disorder centers between September 2014 and July 2016. Patients were suspected of having inherited movement disorders because of early onset, family history, and/or complex phenotypes. They were divided in 5 main movement disorder groups: parkinsonism, dystonia, chorea, paroxysmal movement disorder, and myoclonus. To compare approaches, 23 additional patients suspected of having inherited cerebellar ataxia were included, on whom whole-exome sequencing (WES) was done. Data analysis occurred from November 2015 to October 2016.

MAIN OUTCOMES AND MEASURES Percentages of individuals with positive diagnosis, variants of unknown significance, and negative cases; mutational frequencies and clinical phenotyping of genes associated with movement disorders.

RESULTS Of the 378 patients (of whom 208 were male [55.0%]), and with a median (range) age at disease onset of 31 (0-84) years, probable pathogenic variants were identified in 83 cases (22.0%): 46 patients with parkinsonism (55% of 83 patients), 21 patients (25.3%) with dystonia, 7 patients (8.4%) with chorea, 7 patients (8.4%) with paroxysmal movement disorders, and 2 patients (2.4%) with myoclonus as the predominant phenotype. Some genes were mutated in several cases in the cohort. Patients with pathogenic variants were significantly younger (median age, 27 years; interquartile range [IQR], 5-36 years]) than the patients without diagnosis (median age, 35 years; IQR, 15-46 years; $P = .04$). Diagnostic yield was significantly lower in patients with dystonia (21 of 135; 15.6%; $P = .03$) than in the overall cohort. Unexpected genotype-phenotype correlations in patients with pathogenic variants deviating from the classic phenotype were highlighted, and 49 novel probable pathogenic variants were identified. The WES analysis of the cohort of 23 patients with cerebellar ataxia led to an overall diagnostic yield of 26%, similar to panel analysis but at a cost 6 to 7 times greater.

CONCLUSIONS AND RELEVANCE High-coverage sequencing panel for the delineation of genes associated with movement disorders was efficient and provided a cost-effective diagnostic alternative to whole-exome and whole-genome sequencing.

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Supplemental content

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Movement disorders are a set of heterogeneous neurological syndromes affecting the ability to produce and control movement because of dysfunction in the basal ganglia and/or connected structures.

Movement disorders can be acquired or can arise because of numerous inherited diseases, which are characterized by a great clinical and genetic heterogeneity and a frequent clinical overlap, which prevents reliable genotype-phenotype correlations. The diagnostic work in clinical practice remains a challenging issue, and molecular diagnosis by standard Sanger sequencing is tedious, time consuming, and inefficient, particularly because analysis of the known implicated genes are not yet always routinely available.

The aim of this study was to develop a genetic diagnostic strategy based on a high throughput sequencing technology targeting 127 genes involved in movement disorders. This work intended to assess the efficiency of this approach as a diagnostic tool, help to define mutational frequencies and phenotypic spectra, bring additional evidence for the associations of previous candidate genes with diseases, identify novel mutations, and improve genotype-phenotype correlations.

Key Points

Question Is a customized gene panel study suitable for the identification of genes associated with movement disorders?

Findings This study aimed at developing a targeted sequencing approach using a panel of 127 genes involved in movement disorders and evaluating its performance through analysis of a cohort of 378 patients. A diagnostic yield of 22% was achieved, highlighting some unexpected genotype-phenotype correlations and 49 novel pathogenic variants.

Meaning High-coverage sequencing panel to identify genes associated with movement disorders provided a useful and efficient diagnostic alternative to whole-exome and whole-genome sequencing.

Methods

Study Design

In the multicentric prospective study, patients were selected from 1 Luxembourgian, 1 Algerian, and 25 French tertiary centers specializing in the treatment of movement disorders. Inclusion criteria were that patients (1) had developed 1 or more chronic movement disorders and (2) had an age at onset younger than 40 years and/or a family history of movement disorders. Patients with essential tremor, tic or Tourette syndrome, pure cerebellar ataxia, or clinical or paraclinical findings suggestive of an acquired cause were not included. Most of the patients underwent common biochemical testing (eg, for copper and ceruloplasmin), brain imaging, and most common relevant genetic tests (such as testing for Wilson disease or Huntington disease) before their inclusion. Demographic, clinical, and paraclinical data were collected, as well as family history and a family tree.

The protocol for this study was approved by the Hôpitaux Universitaires de Strasbourg institutional review board. For all patients, a written informed consent for genetic testing was obtained; in the case of minor participants, this was either from adult probands or from a legal representative.

Laboratory Analyses

In the current nonautomated setting of this study, we manually processed each series of 24 DNA samples corresponding to index cases (or series of 6 exomes of index cases) for library preparation, and then libraries were pooled for capture and enrichment reaction. For the sequencing step, we used 1 lane of the Hiseq 4000 Sequencing System (Illumina) flow cell either for the targeted sequencing of 24 samples or the WES of 6 samples. The final step corresponded to the bioinformatics analysis of sequencing data, the interpretation of the variants, and the writing of diagnostic reports. The 127 genes included in the panel are listed in eTable 1 in the Supplement.

Analysis included identification of variants of unknown significance (VUSs). Novel variants were considered as VUSs when they were rare (minor allele frequency < 0.1%) in population databases (eg, Exome Variant Server, Exome Aggregation Consortium, and 1000 Genomes Browser) and/or predicted to be pathogenic by prediction tools (eg, Sorting Intolerant From Tolerant [SIFT], PolyPhen, and MutationTaster), but when the phenotype expressed by the patient was not consistent with the usual phenotype described in the literature.

Statistical Analysis

The diagnostic yield of the movement disorder gene panel was compared with the diagnostic yield of whole-exome sequencing (WES) by performing WES in 23 patients suspected of having autosomal recessive cerebellar ataxia because of early age at onset and/or consanguinity. This analysis was combined with that of previously published WES data on inherited cerebellar ataxias in a sample of 76 patients.[1]

Comparisons between 2 groups were tested for statistical significance using a χ^2 test for qualitative variables and a *t* test for quantitative variables. The Fisher exact test was used in small groups. We considered *P* values of .05 or less statistically significant. For genetic analyses, see eMethods in the Supplement.

Data analyses were completed with the Polyweb interface (Université Paris Descartes and Institut Imagine). Data analysis took place from November 2015 to October 2016.

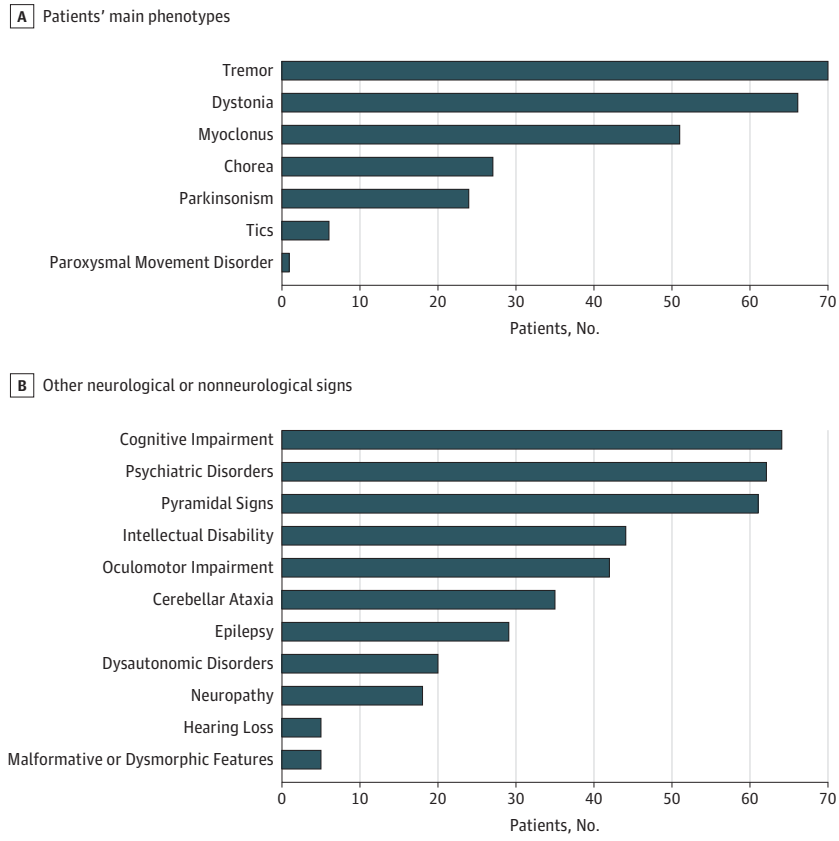
Results

Cohort Description

A total of 378 patients were included in the study, of whom 208 male individuals (55.0%) and 170 female individuals (45.0%). The median age at onset (MAO) of the disease was 31 years (range, 0-84 years). The median age at study inclusion was 45 years (range, 0-87 years). At least 144 patients (38.0%) had undergone genetic testing before their inclusion in the study.

A total of 238 of the 378 patients in the cohort (63.0%) had sporadic cases, and the remaining 140 (37.0%) had a family history of disease. Patients were classified according to their

Figure 1. Clinical Characteristics of Study Participants



Number of patients with other movement disorders associated with the main phenotype (A) and with other neurological or non-neurological signs (B).

prominent movement disorders: parkinsonism (n = 181 of 378 [47.9%]), dystonia (n = 135 [35.7%]), chorea (n = 25 [6.6%]), paroxysmal movement disorders (n = 20 [5.3%]), and myoclonus (n = 17 [4.5%]). Another 166 patients (43.9%) presented with combined movement disorders; this included 12 of the 17 patients with myoclonus (70.6%) and 16 of the 25 patients with chorea (64.0%), as well as 65 of the 135 patients with dystonia (48.1%) and 71 of the 181 patients with parkinsonism (39.2%). Rest tremor was not considered an independent clinical feature in patients with parkinsonism. Associated clinical features are detailed in **Figure 1**.

Diagnostic Yield

Our strategy allowed generating a high-quality sequencing data set, with a mean depth of coverage of 1266 × and a mean of 99.7% of targeted regions being well covered in each patient (× >30).[2] We systematically validated relevant variants identified in the first 48 patients of the cohort and found perfect coherency. Moreover, previously known mutations in the 3 control patients were found by the method. Interestingly, the pipeline seemed to be efficient in copy number variation calling.

We detected pathogenic variants in 83 of 378 patients (**Table**; **Figure 2**, **Figure 3**; eFigure 1 in the Supplement), leading to an overall diagnostic yield of 22.0%. Diagnosis was less frequently established in the dystonia group (n = 21 of 135; 15.6%; $P = .03$) compared with the parkinsonism group (n = 46 of 181; 25.4%; $P = .04$) and the rest of the cohort (n = 62 of 243; 25.5%; $P = .02$). In the 83 patients with genetic diagnoses, the MAO was significantly lower (median, 27 years [IQR, 5-36 years]) than the patients without clear molecular diagnosis (median, 35 years [IQR, 15-46 years]; $P = .04$). In patients with genetic diagnosis, a family history was more frequently found (patients with genetic diagnosis: n = 36 [43.4%] vs patients without genetic diagnosis: n = 103 [34.9%]), as was male predominance (men: n = 50 [60.2%] vs women: n = 156 [52.9%]); however, these comparisons were but without statistical significance. A total of 49 novel probable pathogenic variants were identified.

Seventeen patients harbored variants in *PARKIN*, even though it had already been tested and found negative in 38 members (10.0%) of the entire cohort before inclusion in this study. In addition, 9 patients had variants in *GBA*, and another 8 patients had variants in *LRRK2*. Numerous other very rare entities (with an estimated minor allele frequency of < 0.01%) were identified, such as *PLA2G6* (n = 4), *VPS13A* (n = 4), *ATP13A2* (n = 3), *DNAJC13* (n = 3), *GCH1* (n = 3), *ADCY5* (n = 2), *AP4B1* (n = 2), *DJ-1* (n = 2), *GLRA1* (n = 2), *VPS35* (n = 2), and *WDR45* (n = 2). The rate of identified pathogenic variants was higher in paroxysmal movement disorders (n = 7 of 20; 35%), chorea (n = 7 of 25; 28%), and parkinsonism (n = 46 of 181; 25.4%) than in the cohort overall (n = 83 of 378; 22.0%).

The WES analysis on a cohort of 23 patients with predominantly recessive cerebellar ataxia led to an overall diagnostic yield in 10 patients (43.5%; eTable 2 and eTable 3 in

Table. Probable Pathogenic Variants

Patient No.	Sex	Sporadic/ Familial	Consan-guinity	Movement Disorder	Age at Onset, y	Gene	Mode of Inheritance	Mutations	
								Nomenclature	dbSNP/ClinVar[a]
PMD13	M	Sporadic	Nd	Dystonia	7	*ADCY5*	AD	L720P; htz	Not reported
PMD144	M	Sporadic	No	Chorea	0	*ADCY5*	AD	R418Q; htz	Not reported
PMD403	M	Sporadic	No	Myoclonus	20	*ATP1A3*	AD	N321H/N334H; htz	Not reported
PMD163	M	Familial	No	Parkinsonism	60	*DNAJC13*	AD	R1165G; htz	Not reported
PMD181	F	Familial	No	Parkinsonism	60	*DNAJC13*	AD	K925Q; htz	Not reported
PMD280	F	Familial	No	Parkinsonism	56	*DNAJC13*	AD	K925Q; htz	Not reported
PMD344	M	Sporadic	No	Parkinsonism	38	*EIF4G1*	AD	R1212H; htz	Risk factor
PMD234	F	Familial	Nd	Parkinsonism	39	*GBA*	AD	R398*; htz	Pathogenic[b]
PMD235	M	Familial	Nd	Parkinsonism	55	*GBA*	AD	L483P; htz	Risk factor
PMD238	M	Familial	No	Parkinsonism	51	*GBA*	AD	DelY402; htz	Not reported
PMD279	M	Familial	No	Parkinsonism	43	*GBA*	AD	R502H; htz	Pathogenic*[d]
PMD331	M	Sporadic	No	Parkinsonism	39	*GBA*	AD	W223R; htz	Pathogenic[b]
PMD419	M	Sporadic	No	Parkinsonism	56	*GBA*	AD	L483P; htz	Risk factor
PMD430	M	Sporadic	No	Parkinsonism	39	*GBA*	AD	S235P; htz	Pathogenic[b]
PMD20	F	Familial	Nd	Dystonia	22	*GCH1*	AD	K224R; htz	Pathogenic
PMD138	F	Familial	No	Dystonia	7	*GCH1*	AD	E84K; htz	Not reported
PMD303	M	Sporadic	No	Dystonia	8	*GCH1*	AD	c.343 + 1G→A; htz	Not reported
PMD94	F	Familial	No	Paroxysmal movement disorder	0	*GLRA1*	AD	R299Q; htz	Pathogenic
PMD95	F	Familial	No	Paroxysmal movement disorder	0	*GLRA1*	AD	R299Q; htz	Pathogenic
PMD85	F	Sporadic	Nd	Dystonia	Nd	*GNAL*	AD	c.911-2A→G; htz	Not reported
PMD52	F	Sporadic	No	Parkinsonism	39	*HTRA2*	AD	V200M; htz	Not reported
PMD80	M	Familial	No	Parkinsonism	62	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD81	M	Familial	No	Parkinsonism	68	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD115	M	Sporadic	No	Parkinsonism	35	*LRRK2*	AD	L448S; htz	Not reported
PMD157	F	Sporadic	Nd	Parkinsonism	Nd	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD332	M	Sporadic	No	Parkinsonism	37	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD341	M	Sporadic	Yes	Parkinsonism	70	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD368	M	Sporadic	No	Parkinsonism	22	*LRRK2*	AD	G2019S; htz	Pathogenic
PMD343	M	Sporadic	No	Parkinsonism	38	*LRRK2*	AD	L1114L; htz	Pathogenic
PMD290	M	Familial	No	Dystonia	2	*NKX2-1*	AD	Q249X; htz	Pathogenic
PMD299	F	Sporadic	No	Dystonia	4	*PRKCG*	AD	H347R; htz	Not reported
PMD29	M	Sporadic	No	Paroxysmal movement disorder	5	*PRRT2*	AD	R217Pfs*8; htz	Pathogenic
PMD293	F	Sporadic	No	Paroxysmal movement disorder	8	*PRRT2*	AD	R217Pfs*8; htz	Pathogenic
PMD415	F	Familial	No	Paroxysmal movement disorder	15	*PRRT2*	AD	R217Pfs*8; htz	Pathogenic
PMD201	F	Sporadic	No	Dystonia	0	*SLC2A1*	AD	Q282*; htz	Not reported
PMD211	M	Familial	No	Parkinsonism	68	*SNCA*	AD	Gene duplication	Pathogenic
PMD96	F	Sporadic	No	Dystonia	9	*TOR1A*	AD	E303−; htz	Pathogenic
PMD251	M	Sporadic	No	Dystonia	10	*TUBB4A*	AD	Duplication of exon 4	Not reported
PMD79	M	Familial	No	Parkinsonism	55	*VPS35*	AD	R365C; htz	Not reported
PMD177	F	Sporadic	No	Parkinsonism	69	*VPS35*	AD	H565Q; htz	Not reported
PMD108	M	Sporadic	No	Chorea	7	*ALDH5A1*	AR	G281E; ho	Not reported
PMD75	F	Familial	No	Paroxysmal movement disorder	5	*AP4B1*	AR	R393*; ho	Not reported
PMD76	F	Familial	No	Paroxysmal movement disorder	5	*AP4B1*	AR	R393*; ho	Not reported
PMD54	F	Sporadic	No	Myoclonus	32	*ATP13A2*	AR	F182V; htz	Not reported
								L1053Vfs*60; htz	Not reported
PMD60	F	Familial	No	Parkinsonism	18	*ATP13A2*	AR	G504R; ho	Pathogenic

(continued)

Table. Probable Pathogenic Variants (continued)

Patient No.	Sex	Sporadic/ Familial	Consan-guinity	Movement Disorder	Age at Onset, y	Gene	Mode of Inheritance	Mutations	
								Nomenclature	dbSNP/ClinVar[a]
PMD202	M	Sporadic	No	Dystonia	31	*ATP13A2*	AR	I832V; htz	Not reported
								K804K; htz	Not reported
PMD106	M	Sporadic	No	Parkinsonism	23	*DJ-1*	AR	G157E; htz	Not reported
								Deletion of exon 4; htz	Not reported
PMD148	M	Familial	No	Parkinsonism	31	*DJ-1*	AR	T154A; ho	Not reported
PMD34	F	Sporadic	No	Parkinsonism	5	*PARKIN*	AR	R275W; htz	Pathogenic
								Deletion of exons 3, 4, and 5; htz	Not reported
PMD38	M	Sporadic	Yes	Parkinsonism	16	*PARKIN*	AR	Q34Gfs*5; htz	Not reported
								Deletion of exon 4; htz	Pathogenic
PMD82	F	Familial	Yes	Dystonia	23	*PARKIN*	AR	Q34Gfs*5;ho	Not reported
PMD83	M	Familial	Yes	Dystonia	14	*PARKIN*	AR	Q34Gfs*5; ho	Not reported
PMD116	F	Sporadic	No	Parkinsonism	35	*PARKIN*	AR	N52−; ho	Not reported
PMD132	M	Familial	No	Parkinsonism	13	*PARKIN*	AR	Deletion of exon 5 through 9; htz	Not reported
								Duplication of exon 4; htz	Pathogenic
PMD146	M	Sporadic	No	Dystonia	20	*PARKIN*	AR	Deletion of exons 2, 3, 4, and 5; htz	Not reported
PMD158	M	Sporadic	No	Parkinsonism	17	*PARKIN*	AR	R275W; htz	Pathogenic
								Deletion of exon 4; htz	Pathogenic
PMD214	M	Sporadic	No	Parkinsonism	36	*PARKIN*	AD	R275W; htz	Pathogenic
PMD215	M	Sporadic	No	Parkinsonism	37	*PARKIN*	AD	T240M; htz	Pathogenic
PMD272	F	Familial	Probable	Parkinsonism	27	*PARKIN*	AR	Deletion of exon 4; ho	Pathogenic
PMD273	M	Familial	No	Parkinsonism	49	*PARKIN*	AR	Deletion of exons 2, 3, and 4 ; htz	Not reported
PMD283	M	Familial	No	Parkinsonism	40	*PARKIN*	AR	Deletion of exon 2; htz	Pathogenic
								Deletion of exons 5 and 6; htz	Pathogenic
PMD287	F	Sporadic	Nd	Parkinsonism	60	*PARKIN*	AD	Deletion of exon 4; htz	Pathogenic
PMD337	M	Sporadic	No	Dystonia	33	*PARKIN*	AR	Deletion of exons 3 and 4; htz	Not reported
PMD338	M	Familial	Nd	Parkinsonism	58	*PARKIN*	AR	Q34Gfs*5; htz	Not reported
								R275W; htz	Pathogenic
PMD402	M	Sporadic	No	Parkinsonism	12	*PARKIN*	AR	R275W; htz	Pathogenic
								c.7 + 19G→C; htz	Not reported
PMD364	F	Familial	Yes	Parkinsonism	30	*PINK1*	AR	Q456*; ho	Pathogenic
						LRRK2	AD	G2019S; htz	Pathogenic
PMD9	M	Sporadic	Nd	Parkinsonism	26	*PLA2G6*	AR	G253V; htz	Not reported
								A781T; htz	Reported[c]
PMD16	M	Familial	Nd	Parkinsonism	22	*PLA2G6*	AR	R37*; htz	Pathogenic
								S774I; htz	Not reported
PMD89	F	Sporadic	No	Parkinsonism	3	*PLA2G6*	AR	S504L; htz	Not reported
								Y790*; htz	Pathogenic
PMD365	F	Familial	No	Parkinsonism	41	*PLA2G6*	AR	InsGR516; htz	Not reported
								c.956C→T; htz	Reported[c]
PMD396	F	Familial	Yes	Dystonia	6	*PRKRA*	AR	P222L; ho	Pathogenic
PMD397	F	Familial	Yes	Dystonia	3	*PRKRA*	AR	P222L; ho	Pathogenic
PMD72	M	Sporadic	Yes	Dystonia	2	*SPG11*	AR	L484W; htz	Not reported
								T485Qfs*7; htz	Not reported
PMD73	M	Sporadic	Yes	Dystonia	2	*SUOX*	AR	R459Q; ho	Not reported
PMD22	F	Sporadic	No	Chorea	27	*VPS13A*	AR	T1377Mfs*3; ho	Not reported
PMD63	M	Familial	Yes	Chorea	30	*VPS13A*	AR	Deletion of exon 72; ho	Not reported
PMD117	M	Familial	Probable	Chorea	40	*VPS13A*	AR	Deletion of exon 23; ho	Not reported

(continued)

Table. Probable Pathogenic Variants (continued)

Patient No.	Sex	Sporadic/ Familial	Consanguinity	Movement Disorder	Age at Onset, y	Gene	Mode of Inheritance	Mutations	
								Nomenclature	dbSNP/ClinVar[a]
PMD414	M	Familial	Yes	Chorea	29	*VPS13A*	AR	R3037*; ho	Not reported
PMD53	F	Sporadic	No	Dystonia	1	*MECP2*	XL	R145C; htz	Pathogenic
PMD165	M	Sporadic	No	Parkinsonism	36	*WDR45*	XL	DelQ223/DelQ212; hemz; mosaic	Not reported
PMD369	F	Sporadic	No	Parkinsonism	27	*WDR45*	XL	c.860 + 2T→G/c.827 + 2T→G; htz	Not reported
PMD298	M	Sporadic	No	Dystonia	2	*TIMM8A*	XL	c.132 + 1G→C; hemz	Not reported
PMD395	M	Sporadic	No	Chorea	Nd	*XK*	XL	A270V; hemz	Not reported

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; dbSNP, Single Nucleotide Polymorphism Database; F, female; hemz, hemizygous; ho, homozygous; htz, heterozygous; M, male; Nd, not determined; XL, X-linked.

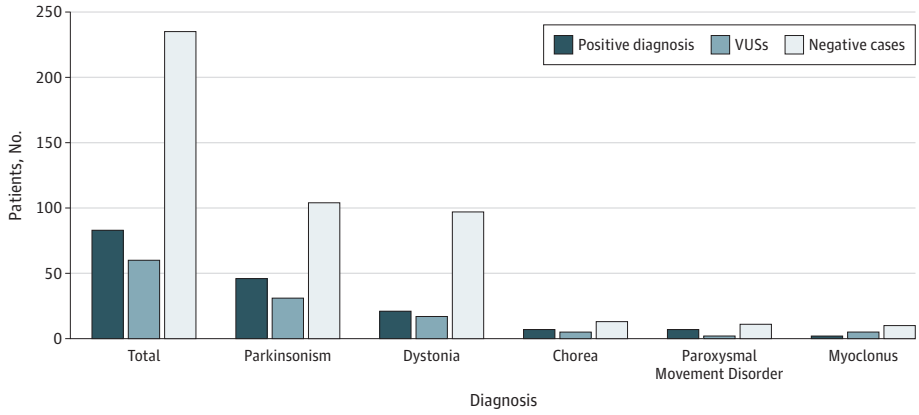
[a] This column reports whether the variant found is reported in the Single Nucleotide Polymorphism Database and/or the ClinVar archive.

[b] Reporting is specific to Gaucher disease.

[c] Never reported at a homozygous state either in Exome Aggregation Consortium or 1000 Genomes Browser.

[d] Reported as a risk factor by Neumann et al.[3]

Figure 2. Diagnostic Yield



Proportion of patients with identified probable pathogenic variants (positive diagnosis), variants of unknown significance (VUSs), and negative cases.

the Supplement). To get significant diagnostic yield for the WES study, our results were combined with the published series of Fogel et al,[1] who obtained a yield of diagnostic pathogenic gene variants in 16 patients in a sample of 76 (21.1%) for a similar series of cerebellar ataxia patients and WES analysis strategy. The overall diagnostic yield for pathogenic gene variants by WES in cerebellar ataxia is therefore 26 of 99 (26.3%), which is similar to the panel approach for movement disorders.

Negative Results

No relevant variants were found in 244 patients (64.6%), with no significant difference between phenotype groups (Figure 2). Their characteristics were similar to the initial cohort, with an MAO of 30 years (IQR, 12-44 years), a proportion of 54.1% male probands (n = 132 of 244), and a proportion of 38.1% familial cases (n = 93 of 244), with family trees suggesting mendelian inheritance (eFigure 2 in the Supplement). These characteristics were not statistically different than the initial cohort.

Variants of Unknown Significance

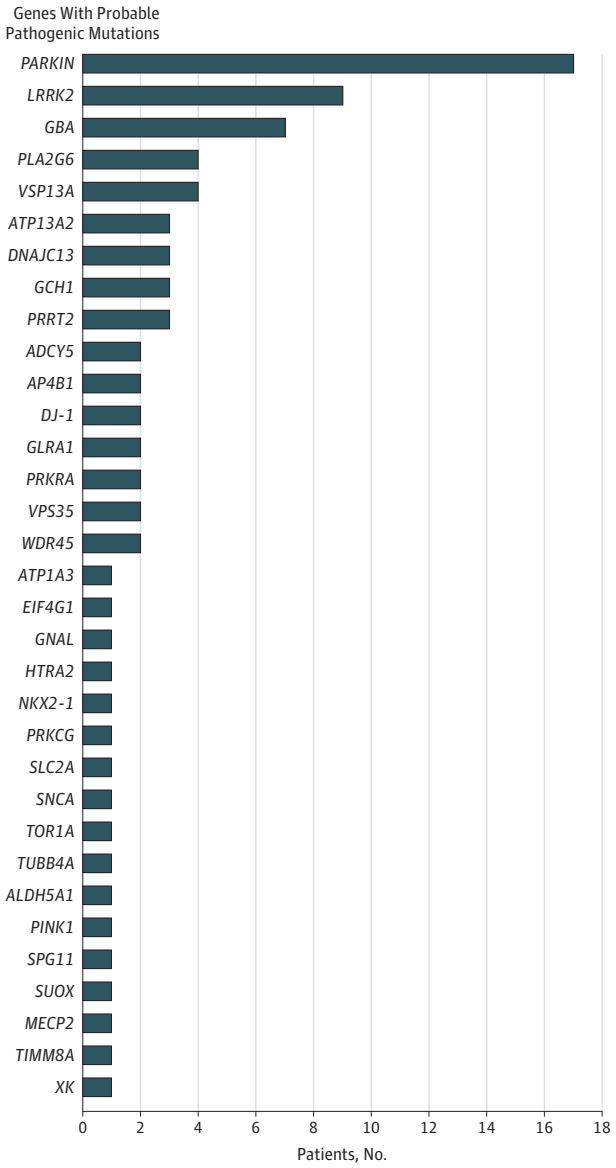
A total of 74 VUSs, which have implications in movement disorder that remain doubtful and must be further studied, were identified in 60 other patients (15.9%; eTable 4 and eFigure 3 in the Supplement; Figure 2). Of the 74 VUSs, 69 (93.2%) were identified in an heterozygous or hemizygous state, and 5 (6.8%) in a homozygous or compound heterozygous state. The age at disease onset, sex, and proportion of family history in patients with VUSs did not significantly differ from the overall cohort.

Peculiar Clinical Findings

In 4 patients, the frameshift deletion c.101_102delAG; Q34Gfs*5 (34Q–) was identified in *PARKIN* in a homozygous or compound heterozygous state. In this cohort, when found at compound heterozygous state, it was associated either with a juvenile-onset dystonia-parkinsonism phenotype or with a typical Parkinson disease presentation. Interestingly, in 2 patients (consanguineous siblings of Algerian descent) who were harboring the homozygous variant, the only symptom was an exercise-induced transient lower-limb dystonia, 10 years after disease onset.

We found pathogenic variants in 46 of 181 patients (25.4%) with parkinsonism. The MAO in patients with heterozygous variants was 44 years, vs 27 years in patients with homozygous or compound heterozygous variants. In our cohort, *LRRK2* variants were identified in 9 patients, including 7 G2019S mutations, 1 L1114L variant (described as pathogenic by Zimprich at al[4]), and 1 novel L448S variant (which was deemed

Figure 3. Distribution of Genetic Diagnoses in the Cohort



Number of patients with probable pathogenic mutations in the specified genes.

probably causative). All patients were presenting with typical Parkinson disease. Interestingly, the MAO was less than 40 years in 6 patients, and cognitive impairment was found in 1 patient. In this study, 8 patients had heterozygous GBA variations, 5 of whom developed typical Parkinson disease, with an age at onset of less than 40 years in 3 patients. Among these 5 patients with Parkinson disease, 2 had either the L483P or the R502H variants, which have already been identified as risk factors for Parkinson disease, and 3 had novel variants: R398*, W223R, or S235P. Two heterogenous missense mutations were identified in *DNAJC13,* in 1 patient experiencing Parkinson disease and cervical dystonia with an age at onset of 60 years (variant R1165G) and in 2 affected siblings with Parkinson disease with ages at onset of 56 and 60 years (variant K925Q). One missense mutation of *HTRA2* has been identified in 1 patient with typical Parkinson disease. Interestingly, 1 hemizygous somatic mosaic point mutation was found in *WDR45* in a male patient with intellectual disability, sporadic early-onset parkinsonism, and T2-weighted magnetic resonance imaging results showing a hyposignal within the substantia nigra. Variants in this gene have been reported as a cause of an X-linked dominant neurodegeneration with brain iron accumulation characterized by childhood developmental disability followed by adolescent or adult onset of dystonia, parkinsonism, and dementia[5] (eResults in the Supplement). These results supported the implication of *DNAJC13* and *HTRA2* in Parkinson disease.

In patients with dystonia, causative variants were identified in only 21 of 135 cases (15.6%). We found 2 consanguineous sisters with childhood-onset myoclonus dystonia harboring homozygous pathogenic variant P222L in *PRKRA*, a gene that has been previously identified in very rare recessive early-onset progressive limb dystonia, laryngeal and oromandibular dystonia, or dystonia-parkinsonism.[6] Unexpectedly, we identified a pathogenic variant in *MECP2* in a 50-year-old female patient who had presented with an undiagnosed mild form of Rett syndrome combined with cerebellar ataxia and dystonic tremor.[7] In addition, a 19-year-old patient was diagnosed with a mild form of sulfite oxidase deficiency (*SUOX*),[8] and an *SLC2A1* variant was found in 1 patient, aged 29 years, who was presenting with delayed psychomotor development, intellectual disability, atonic absence seizures, permanent axial and lower limb dystonia, pyramidal signs, action myoclonus, and microcephaly (eResults in the Supplement).

Regarding cervical dystonia, no relevant variants were identified in genes that were recently identified in WES-based studies (for instance, *CIZ1* and *ANO3*).[9,10] However, 1 patient harbored a variant in *TUBB4A*, and a second patient harbored a variant in *GCH1*.

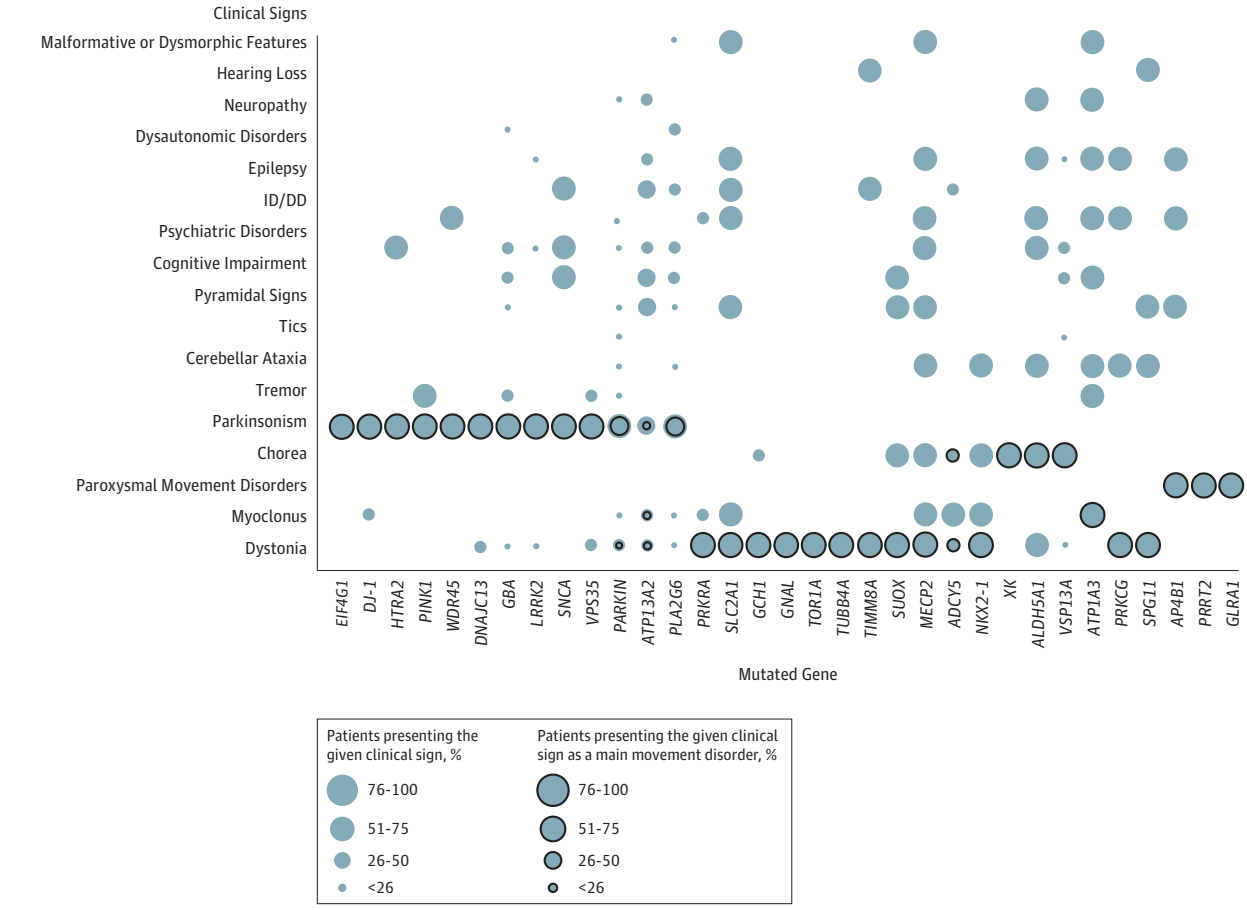
Considering chorea, probable pathogenic variants were found in 7 of 25 patients (28%), in *ADCY5, ALDH5A1, VPS13A* and *XK*, which are known to be involved in chorea. In the study cohort, the 2 patients harboring *ADCY5* variants presented with infantile-onset chorea, facial myokymia, and action myoclonus in 1 case and with childhood-onset generalized dystonia associated with action myoclonus but also with vertical gaze palsy in the second case. In this case, the presentation initially led clinicians to suspect Niemann-Pick disease type C.

Strikingly, causative variants were identified in 7 of 20 patients (35%) with paroxysmal movement disorders. Homozygous *AP4B1* variants were found in 2 sisters with infantile-onset developmental delay, epilepsy, paroxysmal movement disorders (eg, myoclonus), and spastic paraplegia associated with dysmorphic features and a thin corpus callosum.

In 2 of the 17 patients (12%) who presented with myoclonus as a predominant movement disorder, variants were found in *ATP1A3* and *ATP13A2*. Our findings also emphasize marked genetic and phenotypical heterogeneities, as well as the overlaps in the field of movement disorders that were highlighted in this series of findings (**Figure 4**).

Several other probable pathogenic variants were identified in this cohort, especially R393* in *AP4B1* and K925Q in *DNAJC13*, which were found respectively in 2 affected mem-

Figure 4. Phenotypes Associated With Probable Pathogenic Variants Identified in Movement Disorders Genes



ID/DD indicates intellectual disability or developmental delay.

bers of a single family. Segregation analysis supported the implication of the variant in *AP4B1* in the 2 affected siblings, but it could not be performed in the family with the *DNAJC13* variant because of the unavailability of the parents' DNA.

In this study, deletions in the exon 25 of *GIGYF2* (the pathogenicity of which is debated[11,12]) were identified in 4 patients (considered negative results; data not shown) who presented very different phenotypes. Three patients were without any parkinsonian feature, with 2 patients presenting instead with generalized dystonia and 1 patient presenting with myoclonus. Relevant pathogenic variants were found in only 1 of 35 patients (3%) presenting with an associated cerebellar ataxia.

Cost Details of Panel and WES Strategies

The estimated cost of the gene panel strategy without labor costs was $156 per patient (reagent cost of $70, run cost of $68, and $18 of consumable items), whereas the cost of WES without labor costs was estimated between $850 and $1113 per patient. The amount of time required for the gene panel strategy for a series of 24 patients varied between 2 to 3 full days, while data analysis of a series of 6 exomes required 7 to 10 days.

Discussion

Diagnostic Yield

Targeted sequencing of 127 genes in a cohort of 378 patients of mostly European descent with unknown etiology of movement disorders led to a conclusive diagnostic yield of 22.0%. This rate of success is in good accordance with other studies using targeted high throughput sequencing in the diagnosis of neurological diseases.[13,14] The cost-effectiveness of gene panel analyses has already been shown in the field of movement disorders.[14] In our series, the rate of molecular diagnosis was significantly higher when the MAO was lower. The high depth of coverage and the smaller portion of poorly covered regions achieved with our strategy ensure a high sensitivity and specificity of detecting pathogenic events in the regions of interest and enable the identification of single-nucleotide variants, indels, but also copy number variants, which remain a challenge in next-generation sequencing.[15] It also showed accuracy for the detection of somatic mutations. However, it remains incapable of detecting triplet repeat expansions, therefore the search for Huntington disease; spinocer-

ebellar ataxia types 1, 2, 3, 6 and 7; fragile X-associated tremor ataxia syndrome; or dentatorubral-pallidoluysian atrophy was impossible. Furthermore, variant ranking could fail to detect relevant variants with incomplete penetrance because of a higher allele frequency in the general population.

The variants in many genes identified in our series were responsible for complex pictures with combined movement disorders. These findings reinforce the interest to use a gene panel devoted to several distinct types of movement disorders, beyond dystonia and parkinsonism, which were the most frequent signs in our cohort.

Mutation Frequencies

Despite the relatively small size of the cohort, this study gives some interesting epidemiological data, including confirmation of the 3 genes most frequent involved in Parkinson disease[16] and the suggestion that they should be more systematically searched for. Less expectedly, numerous other very rare entities were identified. Variants in *ALDH5A1, ATP1A3, DJ-1, DNAJC13, GBA, GCH1, LRRK2, MECP2, NKX2-1, PINK1, PLA2G6, PRKRA, PRRT2, SLC2A1, SUOX, VPS13A,* and *VPS35* could cause a complex picture with several movement disorders. Variants in *ADCY5, ATP13A2,* and *PARKIN* could be responsible for a wider spectrum of phenotypes, with different possible prominent MDs. By contrast, a few genes were associated with a relatively pure phenotype, as *EIF4G1* (Parkinson disease), *GNAL*, *TOR1A* and *TUBB4A* (dystonia), *XK* (chorea), *PRRT2* (paroxysmal kinesigenic dyskinesia), and *GLRA1* (hyperekplexia).

Identification of Novel Pathogenic Variants

To our knowledge, the 34Q– variant of *PARKIN* had never been described in patients with movement disorders before now. Exercise-induced dystonia has already been described as a presenting feature of young-onset Parkinson disease,[17] similar to the phenotype seen in 2 patients in this study, but other signs of the disease usually appeared within the 5 following years, as well as early-onset levodopa-induced dyskinesias, in contrast to their lack of symptoms 10 years after onset.

Genotype-Phenotype Correlations and Impact on Clinical Practice

It is remarkable that the diagnostic yield in choreic patients was quite high (28%), although Huntington disease, which is known to be the most frequent inherited cause of chorea, could not be diagnosed with the gene panel. These results supported the implication of *DNAJC13* and *HTRA2* in Parkinson disease, 2 genes whose implication has recently been reported and remains debated.[18,19]

Interestingly, the rate of identified pathogenic variants was significantly lower in dystonia (16%), although most of the targeted genes (69 of 127) were associated with dystonia as the prominent clinical sign of disease. The data suggest that *PRKRA* should be added to the genes tested in patients with myoclonus dystonia. With respect to cervical dystonia, 1 patient had had a good response to levodopa, suggesting that a trial dopaminergic treatment might be tested in patients with isolated cervical dystonia. In addition, neurological affections which are more frequently encountered in pediatric patients were identified in 3 adults with dystonia: sulfite oxidase deficiency (*SUOX*), Rett syndrome (*MECP2*), and GLUT1 deficiency syndrome (*SCL2A1*). These results highlight the fact that these diagnoses should be considered in adult patients. In particular, *SLC2A1* mutations should be searched for in patients with complex movement disorders even when the symptoms are permanent and without exacerbation after fasting, since ketogenic diet is highly effective in reducing clinical features especially when started early in childhood. Moreover, for all these patients, even when there is no specific treatment, a suitable genetic counseling can be provided to the patients and their relatives. A lower diagnostic yield was found in patients displaying myoclonus as main sign of the disease, because of the small number of ataxia genes included in the panel (approximately 10 of 127 included genes).

Susceptibility Genes

The occurrence of deletions in the exon 25 of *GIGYF2* in 3 patients without parkinsonian clinical features is evidence against any specific role of these variants in Parkinson disease. By contrast, several heterozygous pathogenic variants were identified in *PARKIN* in patients with Parkinson disease, supporting the previous assertion that heterozygous pathogenic variants are a genetic susceptibility factor for Parkinson disease.[20] Several copy number variants, which are frequently found in the heterozygous state in *PARKIN*-type of early-onset Parkinson disease[21] were also identified in these patients, as well as missense mutations, with a higher mutational frequency than in population databases. The MAO was consistent with literature data.[22] Strikingly, L444P and N370S, the 2 most frequent mutations in previous studies, were not found in this cohort.[3,23-25]

However, 8 VUSs were identified in *LRRK2*. Further studies will be necessary in order to assess whether they are pathogenic.

Comparison of the Diagnostic Yield of Gene Panel With WES in Recessive Ataxia

Though a WES approach enables to explore all genes, WES analysis costs up to 6 to 7 times more than the panel analysis, in addition to being more time-consuming and more likely to lead to the identification of a large number of VUSs. For these reasons, panel analysis seems to be an acceptable alternative for diagnostic setting. The targeted sequencing of 127 genes in this cohort of 378 patients with unknown genetic etiology of movement disorders led to a conclusive diagnostic yield of approximately 25%, which is similar to the overall diagnostic rate by WES for cerebellar ataxia (26%). When restricted to patients with recessive or sporadic cerebellar ataxia and age at onset younger than 20 years, the detection rate increased to 45% in this series (10 of 22), which was similar to the results of Fogel et al[1] for this class of patients (8 of 15 patients [53%]). It therefore appears that efficiency of WES and targeted high-coverage sequencing highly depends on the inclusion criteria, and that broad inclusion criteria that are required in a diagnostic setting result in reduced rate. Moreover, caution should be used for interpretation of the difference between diagnostic yields, as recently reported WES-based stud-

ies on diverse heterogeneous groups of disorders revealed detection rates for confirmed pathogenic mutations ranging from 21% to 41%.[26,27]

Because of the complex genetic and phenotypic heterogeneity of movement disorders, precise molecular diagnosis remains rarely proposed for most patients in France. It is therefore timely to replace direct sequencing strategies consisting in sequentially analyzing candidate genes by more high-throughput next-generation-sequencing-based strategies, such as targeted sequencing of up to several hundred of genes, or WES. Strategies based on WES are very attractive because of their possible application whatever the group of disorders and the clinical features. However, in diagnostic settings, mean coverage and depth usually provided by WES are often considered insufficient for detection of mosaic mutations. Also, it is well established that a WES approach frequently leads to the identification of numerous VUSs or conflicting interpretations. Such findings usually lead to time-consuming research and literature review activity to structure objective opinions and interpretations that are not necessarily translated and delivered to concerned families as diagnostic results.

For these reasons, targeted sequencing appears more appealing for diagnostic settings, and with an even broader panel of genes that includes (for instance) genes involved in ataxia and spastic paraplegia, a higher resulting diagnostic yield could be expected. The high depth of coverage and the smaller portion of poorly covered regions achieved with our strategy ensure a high sensitivity and specificity of detecting pathogenic events in the regions of interest (single-nucleotide variants, indels, but also copy number variants), an issue that remains difficult to address in WES-based studies. Nonetheless, one of the limitations of targeted sequencing is that analysis of newly identified genes is difficult unless diagnostic laboratory can afford regular revision and implementation of renewed and adapted panels. Moreover, targeted sequencing of movement disorder–associated genes offers the possibility of applying such tests to a high proportion of patients awaiting molecular diagnosis, given the significantly lower cost of sequencing, amount of time dedicated for data analysis and interpretation.

With respect to the cost of targeted high-coverage sequencing of movement disorder–associated genes, continuous efforts are being made to optimize the procedure and reduce costs while preserving the quality. In our current setting, consumable for the processing and sequencing of 1 DNA sample is as low as $120, while WES cost varies between $834 to $1200.

The amount of time required for WES strategy was 3 to 4 times higher than for the gene panel strategy, and the panel analysis is moreover less complicated. Of course, the amount of time dedicated to data analysis could be significantly reduced if a WES approach is applied for trios of patients and parents. However, this strategy is often hampered by the availability of the DNA of the parents and by significant additional cost, limiting its use. After reevaluation of clinical and familial criteria (eg, age at disease onset, severity, and family history), we believe that 38 of 244 patients for whom no relevant mutation was identified would need to be explored by WES, preferably through a trio strategy.

Limitations

This study had several limitations. Most patients were European in origin; however, even if the patients included in the present study were from multiple racial/ethnic origins, one would have to be careful when extrapolating the results to other populations because of variability of mutation frequencies and founder effects. In addition, this study is subject to biases because of the small number of patients in each group, the great variability in previous analyses and data collection, the questionable choice of the main movement disorder in many patients, and the fact that dystonia was a frequent cause of inclusion.

As in many other studies based on targeted approaches,[28] the substantial proportion of patients of our investigated cohort that remain without molecular diagnosis raises several hypotheses. Among them, there are the hypothesis of potential additional movement disorder–associated genes that remain to be discovered, the possibility of missed nonexplored mutations located in noncoding regions, and lastly whether more complex genetic patterns involving for example variants with reduced penetrance and oligogenic and/or multifactorial modes of inheritance.[29-31]

Conclusions

In conclusion, this high-throughput sequencing strategy targeting 127 genes appears as a highly efficient, cost-effective diagnostic tool in the field of early-onset or familial movement disorders. Given the inconstant genotype-phenotype correlations, the frequency of combined movement disorders, and the overlaps, the relevance of targeted sequencing as a first intention test for the diagnosis of movement disorders should be considered. It would be interesting to investigate a larger cohort and to improve the design of the targeted regions, especially adding more genes causing cerebellar ataxia, and complete the study by WES analysis on unresolved cases to search for variants in recently identified new genes or in novel candidate genes.

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