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### ► To cite this version:

Thomas Wirth, Christine Tranchant, Nathalie Drouot, Boris Keren, Cyril Mignot, et al.. Increased diagnostic yield in complex dystonia through exome sequencing. *Parkinsonism & Related Disorders*, 2020, 74, pp.50 - 56. 10.1016/j.parkreldis.2020.04.003 . hal-03490916

**HAL Id: hal-03490916**

**<https://hal.science/hal-03490916>**

Submitted on 22 Aug 2022

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## **Increased diagnostic yield in complex dystonia through exome sequencing**

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- **Manuscript words count: 1722 words**
- **Number of figure : 0**
- **Number of tables : 2**
- **Number of references : 12**
- **Supplemental data: yes (4 tables, 1 figure)**

**Keywords:**

- 1) Dystonia
- 2) Next Generation Sequencing
- 3) Genetic diagnosis
- 4) Phenotype
- 5) Exome

## ABSTRACT

**Introduction:** A strategy based on targeted gene panel sequencing identifies possibly pathogenic variants in fewer than 20% of cases in early-onset and familial form of dystonia. By using Whole Exome Sequencing (WES), we aimed to identify the missing genetic causes in dystonic patients without diagnosis despite gene panel sequencing.

**Material and Methods:** WES was applied to DNA samples from 32 patients with early-onset or familial dystonia investigated by sequencing of a 127 movement disorders-associated gene panel. Dystonia was described according to the familial history, body distribution, evolution pattern, age of onset, associated symptoms and associated movement disorders. Rate of diagnoses was evaluated for each clinical feature.

**Results:** We identified causative variants for 11 patients from 9 families in *CTNNA1*, *SUCLG1*, *NUS1*, *CNTNAP1*, *KCNB1*, *RELN*, *GNAO1*, *HIBCH*, *ADCK3* genes, yielding an overall diagnostic rate of 34.4%. Diagnostic yield was higher in complex dystonia compared to non-complex dystonia (66.7%-5.9%;  $p<0.002$ ), especially in patients showing intellectual disability compared to the patients without intellectual disability (87.5%-16.7%;  $p<0.002$ ).

**Conclusion:** Our approach suggests WES as an efficient tool to improve the diagnostic yield after gene panel sequencing in dystonia. Larger study are warranted to confirm a potential genetic overlap between neurodevelopmental diseases and dystonia.

## **INTRODUCTION**

Dystonia displays a high level of clinical heterogeneity and can be associated with other clinical features to form complex phenotypes [1]. Due to the many genes reported in genetic forms of dystonia, molecular diagnosis is most of the time challenging. By assessing a 127 movement disorders (MDs) gene panel including 69 genes responsible for dystonia on a cohort of patients with early-onset or familial movement disorders, we showed that the diagnostic rate was significantly lower in patients experiencing dystonia as prominent symptom than in other movement disorders [2]. Since Whole Exome Sequencing (WES) was shown to improve the diagnostic yield following targeted gene panel sequencing in epileptic encephalopathy [3], we aimed to apply this strategy to identify the missing genetic causes in patients showing familial and/or early-onset dystonia without diagnosis following previous analysis with our 127 MDs gene panel.

## **MATERIAL AND METHODS**

### **Subjects**

The inclusion criteria were the following: i. patients with a phenotype characterized by prominent dystonia with an early-onset (defined as dystonia occurring before the age of 40) or with a family history (at least one first degree relative also affected with dystonia); ii. the lack of clinical findings or laboratory and imaging investigations suggestive of an acquired cause of dystonia. The same criteria were previously used to select the dystonic patients included in our gene panel study [2]. Additionally we required for WES analysis: iii. the lack of genetic diagnosis following a 127 movement disorders gene panel analysis iv. availability of DNA samples from the patient and his two parents in sporadic and familial cases consistent with de novo event or autosomal recessive inheritance, or from at least two related patients coming

from two different generations in familial cases consistent with autosomal dominant inheritance.

All patients and asymptomatic family members were previously examined by a neurologist or a pediatrician experienced in movement disorders. Dystonia was subsequently characterized according to the axis I of the latest consensus for dystonia classification [4]. Dystonia could be classified as isolated or combined with another movement disorder (such as myoclonus, chorea or tic). In addition, following the recent recommendations of the international Parkinson and movement disorder society task force on the nomenclature of genetically determined dystonia [1], dystonia could be classified as complex when associated with relevant symptoms other than movement disorders (such as intellectual disability, epilepsy, ataxia, deafness) in opposition to non-complex dystonia. The study protocol was approved by the University Hospital of Strasbourg, France institutional review board. For all patients and unaffected parents, a written informed consent for genetic testing was obtained prior to WES analysis.

### **Exome sequencing, targeted gene sequencing and variation analysis**

Genomic DNA from peripheral blood samples was extracted according to standard procedures. An exome capture kit (SeqCap EZ Exome probes; Roche-NimbleGen) was used to target all exons. Exons capture was followed by massive parallel 150pb paired-end sequencing (Illumina, San Diego, CA, USA). Read mapping and variant calling were performed following standard bioinformatics procedures [5]. Filtering and prioritization of the variants were conducted using an in house interactive Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67). Variants were filtered according to their frequency (1%) against the dbSNP (<https://www.ncbi.nlm.nih.gov/projects/SNP/>), 1000 Genome Project (<http://www.internationalgenome.org/>), ExAC (<http://exac.broadinstitute.org/>) and GnomAD (<http://gnomad.broadinstitute.org/about>)

databases. In silico prediction of variants pathogenicity was performed using SIFT, PolyPhen and Mutation Taster. Cadd score was also integrated to the prioritization criteria, and a cutoff of 20 was used to determine deleteriousness. Variants were considered as causative if they fulfilled the following criteria: convincing sequencing quality (high number of reads) occurring in genes which are consistent with the phenotype and (1) known disease mutation reported in ClinVar and/or HGMD or (2) loss of function (Lof) variant occurring in genes intolerant to Lof according to the Exac database, or (3) missense variant predicted to be deleterious by at least 2 algorithms, very rare or absent in population databases. Fitting with the model of inheritance was also required to confirm pathogenicity. Variants were also classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines [6]. Causative variants were confirmed by Sanger sequencing. All molecular diagnoses were discussed and validated by referring clinicians.

## **Statistical analysis**

Diagnostic yields were calculated for all clinical characteristics as the ratio of the number of patients presenting the clinical features and diagnosis of interest on the number of all patients presenting the characteristic. Comparisons between two groups were tested for statistical significance using a Fisher's exact for qualitative variable in small groups. Bonferroni correction for multiple comparison test was realized. Given an alpha risk of 0.05,  $P$ -values  $< 0.002$  were considered statistically significant.

## **RESULTS**

### **Cohort description**

Between September 2014 and July 2018, DNA samples from 241 dystonic patients were obtained to perform Targeted Gene Panel analysis [2]. Causative variants were identified in 37 cases (Supplemental Table-1). In the group of 204 dystonic patients without a definite

molecular diagnosis, 32 patients from 24 families fulfilled study inclusion criteria. There was a male predominance in this cohort (sex-ratio 62.5%). Dystonia mean age of onset was 11.9 years, mean age at inclusion was 29 years. Fifteen patients from 7 families had a family history of dystonia and a single patient was born from a consanguineous union (See Supplemental Figure-1 for pedigrees of these families). Detailed phenotypes of these patients are available on Supplemental Table-2.

### **WES findings and rate of diagnosis**

Overall quality of generated sequencing data is illustrated by the average depth of sequencing of 104.16X and the mean coverage of 90.6% (>30X). We identified 11 causative variants in 9 genes, namely *CTNNB1*, *SUCLG1*, *NUS1*, *CNTNAP1*, *KCNB1*, *RELN*, *GNAO1*, *HIBCH*, and *ADCK3*, meeting the pathogenicity criteria in 11 patients from 9 families, establishing a molecular diagnosis for 34.4% of the patients. Pathogenic and likely pathogenic variants identified by WES are provided on Table-1.

We identified a statistically significant higher diagnostic yield in patients presenting with complex dystonia compared to patients with non-complex dystonia (66.7% vs 5.9%;  $p<0.002$ ), especially in patients with intellectual disability compared to patients without intellectual disability (87.50% vs 12.5%;  $p<0.002$ ).

We also observed a trend towards higher diagnostic yield in patients with pyramidal syndrome (75%) infancy-onset dystonia (60%), as compared to patients with a childhood (40%), adolescence (0%), or early-adulthood onset (12.5%). There was also a trend towards higher diagnostic yield in patients with generalized dystonia (66.67%) than in patients with focal (12.5%), multifocal (25%) or segmental dystonia (22.2%). Unlike complex dystonia, familial form of dystonia, combination with movement disorders or progressive course of the



disease were not associated with a higher rate of success. Details about diagnostic yields are provided on Table-2.

Additionally, we identified in the PMD70 family 3 segregating heterozygous missense variants in 3 different candidate genes (*SIP1L1*, *N4BP2*, and *VAMP8*) and a de novo missense variant in *ZNF594* in the PMD570 family, all meeting our pathogenicity criteria. We identified a *GNAOI* variant in the PMD409 family leading to a substitution of an highly conserved amino acid within the adenylate cyclase binding domain, but which was carried by an asymptomatic individual. We also isolated two compound heterozygous missense variants in *FMN2* but which did not meet our pathogenicity criteria (see Supplemental Table-3).

For 14 patients from 11 families, no relevant variants were found.

## DISCUSSION

By using WES, we identified causative variants in known disease-causing genes in 10 out of 24 families included in our study, achieving a molecular diagnosis in 34.4% of patients. This result is close to diagnostic rates provided by WES in dystonia (Supplementary Table-4). Though the growing number of dystonia-related genes might have led to an early obsolescence of the panel which could explain the low diagnostic yield, it is important to mention that most pathogenic variants were identified in cases with complex dystonia especially when dystonia was associated with intellectual disability. No other clinical characteristics were significantly associated with a higher rate of success. Coherently, most of these causative variants were localized in genes previously associated with intellectual disability like *CTNNB1* [7] or *HIBCH* [8], or epileptic encephalopathy such as *KCNB1* [9]. While this statistical result is preliminary and require confirmation in a larger cohort, it suggests a potential genetic and pathophysiological overlap between dystonia and other neurodevelopmental diseases. These genes should be therefore carefully considered during

future dystonia gene panels design, in order to adapt the panels to complex dystonia and improve the rate of diagnosis. This finding also highlights the importance of a careful and comprehensive neurological examination of patients with dystonia selected for WES, so intellectual disability, or other neurological signs could be identified in order to facilitate the interpretation of genetic data.

Our study also has contributed to better delineate the phenotypic spectrum of several dystonia-related genes. De novo mutations in *GNAOI* had previously been isolated in patients showing childhood-onset epileptic encephalopathy or generalized dystonia with developmental delay[10]. Herein we identified a likely pathogenic *GNAOI* missense variants (C215Y) in a family showing milder phenotype consistent with autosomal dominant inherited dystonia. The variation lead to highly conserved amino acid substitution considered to be damaging by all in silico pathogenicity prediction tools. This new mutation is located near previously reported mutations in patients showing prominent hyperkinetic movement disorders such as R209H or R209L. This result suggest that *GNAOI* mutations should be also searched for in autosomal dominant inherited dystonia. Additionally, we identified a de novo missense variant in *RELN* in a patient showing myoclonic-dystonia. While few families showing autosomal dominant myoclonus-dystonia have been reported [11], the association between rare heterozygous *RELN* variant and dystonia remains currently controversial. This finding supports a potential implication of mutations located in this gene in such a phenotype. We also revisit the phenotype of *NUS1*-related encephalopathy to early-onset myoclonic dystonia with intellectual disability but without parkinsonism, epilepsy or cerebellar ataxia [12] thus expanding the spectrum of this dystonia-related gene. This finding also suggest that the clinical spectra of many neurodevelopmental disorder-associated genes are incompletely understood and that dystonia may be more common than currently appreciated. Surprisingly,

we did not identified patients carrying *KMT2B* variant, though it is recognized as one of the most common genetic cause of early-onset generalized dystonia.

To conclude, our work highlights that WES used in an appropriate patient population can increase rate of mutation identification, improve molecular diagnosis of patients with complex dystonia and help to refine the phenotypes related to mutations in genes associated with dystonia or neurodevelopmental diseases.

## **ACKNOWLEDGMENTS**

The authors would like to thank the patients and their families for participation in this study, Bernard JOST from the IGBMC Microarray and Sequencing Platform, the France Parkinson association and the Revue Neurologique for their support.

## **COMPETING INTERESTS**

T.W. received grants from the Revue Neurologique, the Fondation Planiol and the APTES organizations and travels funding from LVL medical; C.T. received honoraria from Allergan and Merz; A.M. received travel funding from Abbvie; E.R received received research support from Merz-Pharma, Orkyn, Aguettant, Elivie, Ipsen, Fondation Desmarest, AMADYS, Agence Nationale de la Recherche and Fonds de Dotation Brou de Laurière; has served on scientific advisory boards for Orkyn, Aguettant, Merz-Pharma and has received honoraria from Orkyn, Aguettant, Merz-Pharma, and Medday-Pharma; S.B. received honoraria from Aguettant and Orkyn and travel funding from Ellivie, Ipsen and Aguettant; V.L. is member of the scientific board of the AFM-Telethon, and received honoraria from PTC Therapeutics, Sarepta Therapeutics, Biogen France SAS, AveXis Inc; M. A. received honoraria from Teva, Merz, AbbVie, Actelion, Johnson and Johnson, Aguettant, Orkyn, LVL, Elivie.

N.D, B.K, C.M., L.C, R.L, L.L-F., A.G., C.L., P.B., O.L-B., M-A.S., M.B., E.O., P.N., D.D., G.R., J.C. declare no conflict of interest.

## FUNDING/SUPPORT

The study has been supported by a grant provided by France Parkinson. Thomas WIRTH was funded by a grant from the Revue Neurologique for this work.

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**Table 1: Causative variants identified by whole exome sequencing**

Identity	Gender	Age of onset	Age at inclusion	Familial history	Phenotype	Brain MRI	inheritance	Gene	Refseq	Variation	POLYPHEN	SIFT	Mutation taster	Cadd score	ACMG criteria	Clinical Significance
PMD50	M	6 months	19 years	No	Multifocal non progressive dystonia, pyramidal syndrome, severe ID	Normal	de novo	CTNNB1	NM_001098209	3_41275708_C_T; c.1603C>T; p.R535*, htz(#)	Probably Damaging (score: -)	Deleterious (score: -)	Disease causing	40	PVS1, PS2	Pathogenic
PMD72	M	2 years	26 years	Consanguinity	Slowly progressive generalized dystonia, pyramidal syndrome, cerebellar syndrome, deafness	T2 basal ganglia hypersignal	AR	SUCLG1	NM_003849	2_84668200_G_C; c.560C>G; p.P187R, ho	Probably Damaging (score: 0.981)	Deleterious (score: 0)	Disease causing	27,9	PM1, PM2, PP2, PP3, PP4	Likely pathogenic
PMD97	M	20 months	5 years	No	Segmental non progressive dystonia of lower limbs without epilepsy, moderate ID	Right pallidum T2 hypersignal	de novo	KCNB1	NM_004975	20_47990871_A_G; c.1226T>C; p.I409T, htz	Probably Damaging (score: 1)	Deleterious (score: 0)	Disease causing	26,5	PS2, PM1, PM2, PP2, PP3	Pathogenic
PMD194	M	7 years	28 years	No	Segmental non progressive dystonia of upper limbs, myoclonus, tremor, mild ID	Normal	de novo	NUS1	NM_138459	6_117997138_G_A; c.305G>A; p.G102D, htz	Probably Damaging (score: 0.951)	Deleterious (score: 0)	Disease causing	29.0	PS2, PM2, PP3	Likely pathogenic
PMD220	M	Birth	5 years	Brother affected	Non progressive generalized dystonia, pyramidal syndrome, profound ID	White matter rarefaction delayed myelination thin corpus callosum	AR (compound)	CNTNAP1	NM_003632	17_40840944_T_C; c.1507T>C; p.F503L, htz	Benign (score: 0.046)	Deleterious (score: 0)	Disease causing	32	PM1, PM2, PP1, PP2, PP3, PP4	Likely pathogenic
										17_40842124_G_A; c.1754G>A; p.C585Y, htz	Probably Damaging (score: 0.969)	Deleterious (score: 0)	Disease causing	24,1	PM1, PM2, PP1, PP2, PP3, PP4	Likely pathogenic
PMD221	M	Birth	9 months	Brother affected	Non progressive generalized dystonia, pyramidal syndrome, profound ID	White matter rarefaction thin corpus callosum	AR (compound)	CNTNAP1	NM_003632	17_40840944_T_C; c.1507T>C; p.F503L, htz	Benign (score: 0.046)	Deleterious (score: 0)	Disease causing	32	PM1, PM2, PP1, PP2, PP3, PP4	Likely pathogenic
										17_40842124_G_A; c.1754G>A; p.C585Y, htz	Probably Damaging (score: 0.969)	Deleterious (score: 0)	Disease causing	24,1	PM1, PM2, PP1, PP2, PP3, PP4	Likely pathogenic
PMD294	M	26 years	29 years	No	Non progressive right upper limb dystonia, myoclonus, epilepsy, pyramidal syndrome, mild ID	Normal	de novo	RELN	NM_005045	7_103191557_A_G; c.6259T>C; W2087R, htz	Probably Damaging (score: 1)	Deleterious (score: 0)	Disease causing	28,6	PS2, PM2, PP3	Likely pathogenic
PMD466	F	12 years	33 years	No	Slowly progressive generalized dystonia, cerebellar ataxia	Cerebellar atrophy	AR (compound)	ADCK3	NM_020247	1_227152967_AC_A ; c.444delC, htz	/	/	Disease causing	/	PVS1, PM2, PP4	Pathogenic
										1_227170667_G_A; c.1012G>A; p.A338T, htz	Probably Damaging (score: 0.988)	Deleterious (score: 0)	Disease causing	25,3	PM1, PM2, PM3, PP3, PP4	Likely pathogenic
PMD479	M	3 years	31 years	Mother affected	Non progressive generalized dystonia, myoclonus, mild ID	Normal	AD	GNAO1	NM_020988	16_56370693_G_A; c.644G>A; p.C215Y, htz	Probably Damaging (score: 0.998)	Deleterious (score: 0)	Disease causing	29,1	PS2, PM1, PM2, PP2, PP3	Pathogenic
PMD479 Mo	F	5 years	66 years	Son affected	Non progressive generalized dystonia, myoclonus, pyramidal syndrome	Not realised	AD	GNAO1	NM_020988	16_56370693_G_A; c.644G>A; p.C215Y, htz	Probably Damaging (score: 0.998)	Deleterious (score: 0)	Disease causing	29,1	PS2, PM1, PM2, PP2, PP3	Pathogenic
PMD495	M	2 years	2 years	No	Paroxysmal right hemidystonia	T2 basal ganglia hypersignal	AR	HIBCH	NM_014362	2_191077780_T_C; c.913A>G; p.T305A, ho (##)	Benign (score: 0.285)	Benign (score: 0.05)	Disease causing	22,8	PS3, PM2, PP2, PP3, PP4	Likely pathogenic

Abbreviations: ho: homozygous, htz: heterozygous, hnz: hemizygous, AR: autosomal recessive, AD: autosomal dominant, compound: compound heterozygous, ACMG : American College of Medical Genetics and Genomics, MIM mendelian Inheritance in Man, (#) reported likely pathogenic [7]; (##) reported pathogenic [8].

**Table 2: Diagnostic rate of success according to the clinical characteristics of dystonia**

	Characteristics		Number of patients	Patients with a genetic diagnosis	Corresponding diagnostic yields	p value
Associated movement disorders	Isolated dystonia		22	7	31.8%	0.70
	Dystonia combined with other movement disorders		10	4	40%	
		Myoclonus	9	4	44.4%	0.68
		Without myoclonus	23	7	30.4%	
		Chorea	1	0	0%	1
		Without chorea	31	11	35.5%	
		Tic	1	1	100%	0.34
		Without tic	31	10	32.2%	
Associated clinical features	Non complex dystonia		17	1	5.9%	0.0005
	Complex dystonia associated with other clinical features		15	10	66.7%	
		Intellectual disability	8	7	87.5%	0.0007
		Without intellectual disability	24	4	16.7%	
		Pyramidal syndrome	8	6	75%	0.009
		Without pyramidal syndrome	24	5	20.8%	
		Cerebellar ataxia	2	2	100%	0.11
		Without cerebellar ataxia	30	9	30%	
		Deafness	1	1	100%	0.34
		Without deafness	31	10	32.2%	
		Epilepsy	1	1	100%	0.34
		Without epilepsy	31	10	32.2%	
		Oculomotor impairment	3	0	0%	0.53
		Without oculomotor impairment	29	11	37.9%	
		Growth delay	1	0	0%	1
		Without growth delay	31	11	35.5%	
Body distribution	Focal		8	1	12.5%	0.21
	Non-focal		24	10	41.6%	
	Segmental		9	2	22.2%	0.42
	Non-segmental		23	9	39.1%	
	Multifocal		4	1	25%	1
	Non-multifocal		28	10	35.7%	
	Generalized		9	6	66.7%	0.035
	Non-generalized		23	5	21.7%	
	Hemidystonia		2	1	50%	1
Non-hemidystonia		30	10	33.3%		
Temporal pattern	Progressive		6	2	33.3%	1
	Non progressive		26	9	34.6%	
	Paroxysmal		2	1	50%	1
	Non paroxysmal		30	10	33.3%	
Age of onset	Infancy 0-2		10	6	60%	0.06
	Onset after 2		22	5	22.7%	
	Childhood 3-12		10	4	40%	0.70
	Onset before 3 or after 12		22	7	31.8%	
	Adolescence 13-20		3	0	0%	0.53
	Onset before 13 or after 20		29	11	37.9%	
	Early adulthood 21-40		8	1	12.5%	0.1
	Onset after 40 or before 21		24	10	41.6%	
	Late adulthood (> 40)		1	0	0%	1
	Onset before 40		31	11	35.5%	
Familial history of dystonia or consanguinity	Absence		16	6	37.5%	1
	Presence		16	5	31.2%	