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A post-hoc study on gene panel analysis for the diagnosis of dystonia

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ABSTRACT

Background: Genetic disorders causing dystonia show great heterogeneity. Recent studies have suggested that next-generation sequencing techniques, such as gene panel analysis, can be effective in diagnosing heterogeneous conditions.

Objectives: We investigated whether dystonia patients with a suspected genetic cause could benefit from the use of gene panel analysis.

Methods: In this post-hoc study, we describe gene panel analysis results of 61 dystonia patients (mean age 31 years, 72% young-onset) in our tertiary referral centre. The panel covered 94 dystonia-associated genes. As comparison with a historic cohort was not possible due to the rapidly growing list of dystonia genes, we compared the diagnostic work-up with and without gene panel analysis in the same patients. The work-up without gene panel analysis (control group) included theoretical diagnostic strategies formulated by independent experts in the field, based on detailed case descriptions. The primary outcome measure was diagnostic yield, secondary measures were cost and duration of diagnostic work-up.

Results: Work-up with gene panel analysis led to a confirmed molecular diagnosis in 14.8%, versus 7.4% in the control group (p=0.096). In the control group on average 3 genes/case were requested. The mean costs were lower in the gene panel analysis group (€1822/case) than in the controls (€2660/case). The duration of work-up was considerably shorter with gene panel analysis (28 vs 102 days).

Conclusion: Gene panel analysis facilitates molecular diagnosis in complex cases of dystonia, with a good diagnostic yield (14.8%), a quicker diagnostic work-up, and lower costs, representing a major improvement for patients and their families.

INTRODUCTION

Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements or postures, or both. Dystonic movements are typically patterned, twisting, and may be tremulous. They are often initiated or worsened by voluntary action and associated with overflow muscle activation.[1] The clinical evaluation of a patient with dystonia is a stepwise process, beginning with classification of the dystonia characteristics according to the latest consensus criteria and recognition of the dystonia syndrome; this, in turn, may lead to a targeted etiological differential diagnosis.[2]

There is a long list of causes of dystonia,[3,4] while clinical clues for a genetic form include a positive family history and young-onset in the absence of an acquired cause.[5] A complex clinical picture comprising both neurological and non-neurological features is considered to be an important clue for an inborn error of metabolism.[6]

The genetic disorders associated with dystonia are often clinically heterogeneous, with milder or atypical phenotypes that may easily remain unrecognized. The diagnostic work-up of dystonia can therefore be challenging and time-consuming, and poses a burden on patients and their families.

It has become possible to analyse thousands of genes simultaneously, since next generation sequencing (NGS) techniques were introduced into clinical diagnostics.[6,7] Several studies suggest that NGS diagnostic strategies can be particularly effective in diagnosing heterogeneous conditions, including movement disorders.[8-11] One of these NGS techniques is targeted gene panel analysis (GPA), which comprises testing a preselected list (or panel) of genes causing dystonia. Compared to other NGS techniques, such as whole genome- and whole exome sequencing, the cost of GPA is lower and it provides a higher coverage and

fewer unsolicited findings. GPA is therefore a good strategy to scan panels of multiple candidate genes and it is especially suitable for diagnostic purposes.[11,12]

Despite a strong tendency to advocate the advantages of NGS testing, there is no evidence as yet that NGS approaches perform better than conventional diagnostic strategies for dystonic patients in clinical practice. In cases with an easily recognizable, classical phenotype, NGS techniques have limited added value so single gene testing is recommended.[9] However, in many dystonia cases in which several potentially causal genes are being considered, it is hypothesized that NGS strategies hold advantages like an earlier diagnosis, a higher diagnostic yield and lower costs.[9,11]

This study therefore aimed to determine the possible benefits of using GPA for dystonia patients with a suspected genetic cause compared to conventional diagnostic work-up.

MATERIALS AND METHODS

Patients

All patients in this study were referred to the tertiary Movement Disorders outpatient clinic of the University Medical Centre Groningen (the Netherlands), to establish the cause of their dystonia. In 2013 we introduced GPA of 94 dystonia-associated genes (Supplement 1) as part of routine clinical DNA diagnostic testing. Patients of all ages were consecutively enrolled in our study if they had isolated dystonia or dystonia as a main symptom, a clinical suspicion of a genetic cause, and genetic testing using GPA that was performed between December 2013 and April 2015. Clinical suspicion of a genetic cause was defined as: absence of clinical clues suggesting an acquired cause of dystonia,[5] in combination with one or more of the following:

onset of dystonia before the age of 40 years; a positive family history; dystonia combined with another movement disorder; co-occurrence of other unexplained neurological or systemic manifestations; paroxysmal dystonia; laryngeal dystonia (also known as “spasmodic dysphonia”). Exclusion criteria were an acquired form of dystonia, no clinical suspicion of a genetic cause and dystonia as a minor feature.

Of the 61 patients enrolled, 28 (46%) were male. Their mean age was 31.0 years (SD 21.8, range 1-73 years) on their first visit to our clinic. 44 (72%) of the patients had young-onset dystonia (starting before age 21 years). The patients’ characteristics are summarized in Table 1 and an overview of the clinical characteristics of each individual patient is provided in Supplement 2.

Gene panel analysis

The genes included in the dystonia GPA (Supplement 1) were selected based on a systematic literature review.[5] From the list of all genes associated with dystonia, genes reported only in single families/cases were not put on the diagnostic panel, this to reduce the potential number of variants needed to be interpreted by genome staff. Therefore, the unconfirmed candidate genes *CACNA1B* and *CIZ1* were omitted from the list. Notably, the list of 94 genes of the gene panel excludes several dystonia-associated genes (for example the spinocerebellar ataxias genes) because GPA cannot detect repeat expansions, whole exon duplications or deletions.

GPA was offered to patients as a clinical diagnostic test, validated by the standards of the Dutch Society for Clinical Genetic Laboratory Diagnostics[13]. Also the interpretation and letters reporting test results were based on these guidelines. All test results, including

pathogenic variants and variants of unknown significance, were first discussed in a multidisciplinary meeting with neurologists, clinical geneticists and genome laboratory staff. When the clinicians stated that a variant of unknown significance in a gene could explain the clinical phenotype, additional diagnostics steps were undertaken, such as array-comparative genomic hybridization (array-CGH), multiplex ligation-dependent probe amplification (MLPA) analysis for autosomal recessive disorders, or sequencing the DNA of the parents to detect de-novo variants for dominant disorders. In some cases biochemical testing was done to confirm a diagnosis.

Study Design

We conducted a post-hoc analysis by comparing the GPA study group to a theoretical control group without GPA. The primary outcome was the diagnostic yield and secondary outcomes were the cost and duration of diagnostic work-up.

As comparison with a historic cohort was not possible due to the rapidly growing list of dystonia genes, we compared the diagnostic work-up with and without GPA in the same patients. Each patient in our study therefore served as his/her own control. We built up a theoretical situation in which independent experts in the field were twice asked to formulate a diagnostic strategy: first based only on the detailed clinical case description (Part 1), and secondly after we incorporated the results of further additional tests, like laboratory investigations and neuroimaging findings, except for the results of GPA (Part 2).

The diagnostic yield obtained in the controls after Part 2 was compared to the diagnostic yield obtained for the patients after GPA. The cost and duration of the diagnostic strategy for the controls in Part 1 were compared to the cost and time required to perform the dystonia GPA. These study methods will be discussed in more detail below. Figure 1 gives an overview of the study design.

Diagnostic evaluation with GPA

For the diagnostic work-up in the study group we followed our reported algorithm.[5] None of our 61 cases had any clinical clues of an acquired form of dystonia, neither in their clinical presentation nor on brain MRI. Biochemical diagnostics and a levodopa trial,[5] were not included in the diagnostic work-up in the study group, as GPA is quicker in our centre (28 days). Therefore, the diagnostic work-up in the study group consisted exclusively of GPA.

Diagnostic evaluation without GPA

For each case, we had a description of the clinical phenotype (comprising the patient's medical history, history of present illness, family history, medication use, the physical and neurological examination findings, and the results of the brain MRI). All case descriptions were reviewed by the treating physician to ensure they presented an accurate reflection of the clinical picture.

We asked eight independent international experts to take part in our study (4 neurologists and 4 paediatric neurologists). Each case description was anonymised and randomly sent to two experts, who independently assessed the cases. We took into account the age of the patient at the time of examination when sending the cases to the paediatric or adult neurologists, using the age of 18 as cut-off point.

Each case would be independently assessed by two experts, and inevitably, both experts would have differences in their assessments. Therefore, we decided to consider each assessment separately, resulting in a control group of 122 assessments.

In Part 1 (control group), the experts formulated their theoretical diagnostic work-up and diagnostic guess. The experts could request any additional tests they deemed necessary, including a levodopa trial and single gene testing, but they were not allowed to use any NGS techniques in their diagnostic work-up. In Part 2 the case descriptions were again given to the same expert, but now with the results of all the additional tests (serum, urine, cerebrospinal fluid (CSF) analysis, muscle and skin biopsies, consultations with other medical specialists, neuroimaging, neurophysiological tests, and the levodopa trial if available), all this information was retrieved from the patients' medical records. However, the results of the dystonia GPA were not provided.

After Part 2, the two experts reported their theoretical diagnostic strategies and diagnostic guess, again independently. They could request any extra tests deemed necessary, except for NGS techniques.

Outcome measures

We defined the diagnostic yield as the percentage of cases with a genetically confirmed diagnosis. The diagnostic yield of the dystonia GPA in the 61 cases of the study group was compared to the diagnostic yield of the 122 theoretical diagnostic strategies in the control group. The diagnostic yield of the control group without GPA was established by assessing whether the single gene testing requested by the experts would have led to the etiological diagnosis according to the results of the dystonia GPA.

We investigated the cost of performing the dystonia GPA at our centre (study group), and also the cost of performing the diagnostic tests requested by the experts for the controls in Part 1 (see Supplement 3).

To establish the duration of the diagnostic work-up, we used standard reporting times for the diagnostic procedures in our clinic (see Supplement 3). In the study group, we defined the duration of diagnostic work-up as the time between requesting the dystonia GPA and receiving the results. In the control group (Part 1), we determined the theoretical cost and theoretical duration of diagnostic work-up based on the experts' proposed strategies. To establish the duration, we considered the sequence of tests requested (simultaneous tests versus sequential tests).When an expert requested multiple simultaneous tests, only the test with the longest duration to generate a diagnostic report was taken into account. If the expert's strategy would have led to an etiological diagnosis halfway through the theoretical procedure the cost and time-frame of the remaining tests were not used in the analysis.

The use of brain MRI was not taken into account in analysing both the study and control groups, because we considered the MRI to be an indispensable part of both the conventional diagnostic work-up (control group) and of the work-up with GPA (study group).[2,5] Therefore, the results of brain MRI were included in the clinical case descriptions in Part 1.

Statistical analysis

We used SPSS (version 22, IBM SPSS Statistics) for our analysis and the one-sided Fisher's Exact Test (α=0.05) for our primary outcome. For the secondary outcomes, we described the mean and range of the cost and duration of the diagnostic work-up, with and without GPA. Comparison with Fisher's Exact test was not possible for the secondary outcomes because of the fixed cost and fixed duration of GPA.

RESULTS

Study group: diagnostic work-up with GPA

In a multidisciplinary meeting with neurologists, clinical geneticists and genome laboratory staff all GPA results were discussed, including pathogenic variants and variants of unknown significance. Population frequencies and conflicting data regarding specific variants known in the literature were taken into consideration. In the series of patients included in this study, on average 0 to 2 variants of unknown significance were detected, and all were considered very unlikely to explain the phenotype. Therefore additional diagnostic steps, such array-CGH or MLPA analysis, were felt unnecessary.

In the study group a genetically confirmed cause of the dystonia was determined in 9/61 patients (14.8%). The following diagnoses were made: DYT16 (*PRKRA* gene), Segawa syndrome (*TH* gene), glutaric aciduria type I (*GCDH* gene), Niemann-Pick type C (*NPC1* gene), paroxysmal kinesigenic dyskinesia (*PRRT2* gene) in three patients, and Rett syndrome (*MECP2* gene) in two patients (Table 2).

Control group: diagnostic work-up without GPA

For the 122 control case descriptions, there was a total of 355 requests for gene tests on 66 different genes. On average, 3 single gene tests per case were requested by the experts. These led to identification of the genetic cause of the dystonia in 9/122 assessments (7.4%), see Table 2.

Diagnostic Yield

The genetic diagnostic yield in the study group with GPA (14.8%) was higher than in the controls without GPA (7.4%), with statistical analysis tending towards, but not reaching, levels of significance (p=0.096).

Cost and duration of diagnosic work-up

The cost of performing the dystonia GPA for one patient was €1,822 (study group). An overview of the requested diagnostic tests in the control group is shown in Supplement 3. The sum total of cost of the diagnostic strategies in the controls was € 324,482.93, which was divided by 122, resulting in a mean cost for the diagnostic tests in the controls of €2,660 per patient (SD 2747, range €0 to €18,688). For an overview of the requested tests in the control group, see Supplement 3.

We performed a sub-analysis to compare the cost of the diagnostic work-up using only single gene testing in the control group, with the cost of GPA. The cost of the work-up with single gene testing alone was € 2,238 per patient (SD 2,444, range €0 to €16,918), which is higher than the cost of GPA (€1,822 per patient).

For the study group, the timeframe between requesting the dystonia GPA and receiving the results was 28 days. The mean duration of the diagnostic work-up in the controls was 102 days (SD 66 days, range 0-301 days).

The lower limit of the range of zero for cost and duration of the diagnostic work-up in the control group was based one case where one of the experts decided not to do any additional testing because of a presumed stationary encephalopathy.

DISCUSSION

This study shows that GPA facilitates molecular diagnosis in complex cases of dystonia, with a good diagnostic yield (14.8%), a quicker diagnostic work-up, and lower costs.

In an ideal situation we would have set up a prospective cohort study, however, in such a study design it would not be ethically justified to withhold the use of NGS diagnostics to patients in the control group. We considered using a historic control group, but using a historic cohort of dystonia patients would not be relevant, as the list of known dystonia genes has expanded rapidly. Therefore, we compared the diagnostic work-up with and without GPA in the same patients: each patient in our study served as his/her own control. The study design reflects a pragmatic approach: we evaluated how dystonia diagnostics are performed in clinical practice, with the aim of helping clinicians to make an informed choice between the conventional diagnostic work-up and a work-up with GPA.

The use of GPA in dystonia diagnostics in this study increased the yield compared to conventional work-up, with statistical analysis tending towards, but not reaching, levels of significance. This may be due to the relatively small group of patients.

Looking more closely at our results, we saw that particularly patients with an unusual or complex phenotype benefitted from GPA, with disorders not considered in the initial differential diagnosis being identified. Below, we highlight three examples from our study.

First, a patient who presented at the age of 44 years in whom GPA analysis demonstrated a *GCDH* gene mutation (glutaric acidemia type I). Second, a patient with adult-onset myoclonus who later developed dystonia in his sixties and proved to have Niemann-Pick type C disease. And third, a patient with motor developmental delay as a child, who

developed rapidly progressive parkinsonism and multifocal dystonia at age 13, and was then found to have a *TH* (Tyrosine Hydroxylase) gene mutation. Importantly, all three disorders are treatable forms of inborn errors of metabolism, with an accurate diagnosis allowing prompt initiation of therapy. Our findings are in line with other studies that suggest that particularly patients with a non-specific or atypical clinical presentation will most likely benefit from NGS diagnostics.[9-11]

To our knowledge, this is the first study comparing the diagnostic yield of NGS techniques to conventional genetic techniques in diagnosing patients with dystonia, although other studies have compared NGS techniques to conventional genetic testing in other disorders, including movement disorders.[8,9]

Neveling and colleagues compared whole exome sequencing (WES) to Sanger sequencing in patients with heterogeneous diseases, including movement disorders.[8] The use of WES in 50 patients with movement disorders (29 hereditary spastic paraplegia, 12 cerebellar ataxia, 9 dystonia) compared to Sanger sequencing in 953 patients with movement disorders: WES had a diagnostic yield of 20% versus 5% in the Sanger sequencing group. The diagnostic yield of NGS in movement disorders in the study of Neveling et al. is higher (20%) than in our study (14%). This can be explained by differences in the patient population tested and a different study design, but another reason may lie in the fact that GPA is restricted to preselected genes only, in contrast to WES. However, when we designed our study, we opted for GPA as the genetic coverage (sequencing depth) was higher than with other forms of NGS, at lower cost and with fewer variants to be interpreted and unsolicited findings.

Notably, the patients included in our study were all tertiary referrals and 16 (26%) of them were referred to us from other tertiary centres (Table 1). As a consequence, our study population comprised many complex cases, which is reflected in the proportion of cases that remained undiagnosed even after GPA. This is in line with other GPA studies with highly selected patient populations.[12,16]

In heterogeneous disorders where several potential genes are considered, the hypothesized advantages of NGS strategies are not only a higher diagnostic yield, but also an earlier diagnosis and lower costs. However, there is little published data relating to the cost-effectiveness of NGS technologies to date.[17,18]

In our study, the mean costs were lower in the GPA group (€1822/case) than in the controls (€2660/case), and the cost incurred per expert varied greatly (range €0 to over €18,988). This illustrates the very different diagnostic strategies used by individual experts. One possible explanation is the variability in costs, budgets and availability of diagnostic procedures between centres and countries, leading to different daily routines of clinicians. The cases with the highest costs were those with the most complex phenotypes, in which the cost-effectiveness of GPA can be highest. This is consistent with the cost-effectiveness of WES recently demonstrated in complex cases in a paediatric cohort with heterogeneous disorders.[18]

The duration of the diagnostic work-up with GPA was considerably shorter than the mean duration of the conventional work-up in the control group (28 vs 102 days). The duration in the control group is likely to be underestimated, as there is usually a delay in clinical practice between receiving the results of investigations, obtaining patient consent for the next diagnostic test, and requesting the next test. Furthermore, it has been shown in other

studies that the diagnostic work-up for dystonia patients may require many years.[5,19] The reason for the relatively short duration of the diagnostic work-up in the control group of our study is probably the involvement of highly experienced dystonia experts. A quicker diagnostic work-up in the GPA group compared to conventional is a relevant finding, because the diagnostic odyssey is costly both in terms of health care resources and poses a burden on the patient and his/her family.[20] In addition, diagnostic delays can have major implications with regard to potential therapies and avoiding unnecessary investigations.

In conclusion, our results show that GPA facilitates molecular diagnosis in complex cases of dystonia, with a good diagnostic yield, a quicker diagnostic work-up, and lower costs, representing a major improvement for patients and their families. However, as Hennekam and Biesecker clearly stated, NGS and computers will not magically make diagnoses for us.[21] Careful clinical evaluation of the patient remains fundamental and NGS should not replace deep clinical phenotyping. As evident from our study, Sanger sequencing of the candidate gene will often lead to a diagnosis in cases with a classical phenotype. In patients with complex and unusual phenotypes careful clinical evaluation remains important as ever, however, in these cases there will be a shift from a pre-NGS-test differential diagnostic mode to a post-NGS-test diagnostic assessment mode.[21] In line with this, a user-friendly and expandable online tool has been developed to help movement disorder clinicians to link NGS-test results to the clinical and phenotypic data of the individual patient.[22]

In the near future, NGS techniques will become increasingly incorporated into our daily clinical routines. Here we choose to use a targeted gene panel analysis, but WES coverage has improved significantly over time at much lower costs, making it more accessible for routine diagnostic purposes. With these advances in WES it will become easier to keep diagnostic tests up-to-date to the rapid expanding lists of genes associated with

dystonia, but also to have the possibility to unravel novel dystonia associated genes. For heterogeneous disorders, such as dystonia, these developments will lead to earlier etiological diagnosis in a higher proportion of cases.

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Authors' Roles:

Martje E. van Egmond: organization and execution of the research project, assessment of the clinical case descriptions, writing of the first draft, review and critique of the manuscript; *Coen H.A. Lugtenberg*: organization and execution of the research project, data analysis, writing of the first draft, review and critique of the manuscript; *Oebele F. Brouwer*: assessment of the clinical case descriptions; review and critique of the manuscript; *Maria Fiorella Contarino*: assessment of the clinical case descriptions; review and critique of the manuscript; *Victor S.C. Fung*: assessment of the clinical case descriptions; review and critique of the manuscript; *M. Rebecca Heiner-Fokkema:* design and validation of gene panel and interpretation of panels results, review and critique of the manuscript; *Jacobus J van Hilten*: review and critique of the manuscript; *Annemarie H. van der Hout*: interpretation of gene panel results, review and critique of the manuscript; *Kathryn J. Peall*: assessment of the

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Legend to Figure 1

Study design scheme, 61 cases were included. Descriptions were made based on the patients' medical records. Two independent experts assessed the cases on their clinical features and developed a theoretical diagnostic strategy for each case (Part 1). They could request any

extra tests deemed necessary, except for next-generation sequencing techniques. In Part 2 the cases were supplemented with the results of additional investigations and reassessed by the experts.

Supplement 1. Dystonia gene panel (94 dystonia-related genes)

Gene (OMIM)	*Disease name/phenotype*	*Mode of inheritance*
ADAR (146920)	Aicardi-Goutieres syndrome 6	AR, AD
ADCY5 (600293)	Familial dyskinesia with facial myokymia	AD
ALDH5A1 (610045)	Succinic semialdehyde dehydrogenase deficiency	AR
ANO3 (610110)	DYT24: isolated focal dystonia	AD
ARX (300382)	Partington syndrome/X-linked mental retardation	XR
ATP13A2 (610513)	Kufor-Rakeb syndrome (PARK9)	AR
ATP1A3 (182350)	DYT12: Rapid-onset dystonia parkinsonism	AD
ATP7B (606882)	Wilson's disease	AR
BCS1L (603647)	Mitochondrial complex III deficiency/Leigh syndrome	AR
*C10ORF2 (*609286)	Progressive external ophtalmoplegia with mitochondrial DNA deletions	AD
C19orf12 (614297)	Neurodegeneration with brain iron accumulation (NBIA) 4	AR
CDKL5 (300203)	Early-onset epileptic encephalopathy / variant Rett syndrome	XD
COX10 (602125)	Mitochondrial complex IV deficiency/Leigh syndrome	AR
COX15 (603646)	Mitochondrial complex IV deficiency/Leigh syndrome	AR
COX20 (614698)	Mitochondrial complex IV deficiency	AR
CP (117700)	Aceruloplasminemia	AR
DDC (107930)	Aromatic L-amino acid decarboxylase deficiency	AR

DJ1 (602533)	Early onset Parkinson disease type 7 (PARK7)	AR
DLAT (608770)	Pyruvate dehydrogenase E2 deficiency	AR
DLD (238331)	Dihydrolipoamide dehydrogenase deficiency (Maple syrup urine disease type III)	AR
FA2H (611026)	Spastic paraplegia type 35	AR
FBXO7 (605648)	Early onset Parkinson disease type 15 (PARK15)	AR
FOLR1 (136430)	Cerebral folate deficiency	AR
FOXG1 (164874)	Rett syndrome, congenital variant	de novo
FTL (134790)	Neurodegeneration with brain iron accumulation (NBIA) 3	AD
FUS (137070)	Hereditary essential tremor (ETM4)	AD
GCDH (608801)	Glutaric aciduria type 1	AR
GCH1 (600225)	DYT5: GTP-cyclohydrolase 1 deficiency	AD
GNAL (139312)	DYT25: isolated dystonia	AD
LRPPRC (607544)	Leigh syndrome, French-Canadian type	AR
MECP2 (300005)	Rett syndrome	XD
MTTP (157147)	Abetalipoproteinemia	AR
NDUFA10 (603835)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFA12 (614530)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFA2 (602137)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFA9 (603834)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFAF2 (609653)		AR

	Mitochondrial complex I deficiency/Leigh syndrome	
NDUFAF5 (612360)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFAF6 (*C8orf38*) (612392)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFS1 (157655)	Mitochondrial complex I deficiency	AR
NDUFS3 (603846)	Mitochondrial complex I deficiency	AR
NDUFS4 (602694)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFS7 (601825)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NDUFS8 (602141)	Mitochondrial complex I deficiency/Leigh syndrome	AR
NKX2-1/TITF1 (600635)	Benign hereditary chorea	AD
NPC1 (607623)	Niemann Pick type C	AR
NPC2 (601015)	Niemann Pick type C	AR
NUP62 (605815)	Infantile striatonigral degeneration	AR
PAH (612349)	Phenylketonuria/hyperphenylalaninemia	AR
PANK2 (606157)	Neurodegeneration with brain iron accumulation (NBIA) 1/HARP	AR
PRKN (602544)	Juvenile Parkinson disease type 2 (PARK2)	AR
PCBD1 (126090)	Hyperphenylalaninemia variant D	AR
PDHA1 (300502)	Pyruvate dehydrogenase E1-alpha deficiency	XD
PDHB (179060)	Pyruvate dehydrogenase E1-beta deficiency	AR
PDHX (608769)	Pyruvate dehydrogenase E3-binding protein deficiency	AR

PINK1 (608309)	Early onset Parkinson disease type 6 (PARK6)	AR
PLA2G6 (603604)	Neurodegeneration with brain iron accumulation (NBIA) 2/PARK14	AR
PLP1 (300401)	Pelizaeus-Merzbacher disease	XR
PNKD/MR1 (609023)	DYT8: Paroxysmal non-kinesigenic dyskinesia	AD
POLG (174763)	Alpers/MNGIE/SANDO (Mitochondrial DNA depletion syndrome 4)	AR
PRKRA (603424)	DYT16: Young-onset dystonia parkinsonism	AR
PRRT2 (614386)	DYT10: Paroxysmal kinesigenic dyskinesia	AD
PTS (612719)	6-Pyruvoyltetra-hydropterin synthase (PTPS) deficiency	AR
QDPR (612676)	Dihydropteridine reductase (DHPR) deficiency	AR
RNASEH2A (606034)	Aicardi-Goutieres syndrome 4	AR
RNASEH2B (610362)	Aicardi-Goutieres syndrome 2	AR
RNASEH2C (610330)	Aicardi-Goutieres syndrome 3	AR
SAMHD1 (606754)	Aicardi-Goutieres syndrome 5	AR
SCO2 (604272)	Cardioencephalomyopathy due to cytochrome c oxidase deficiency 1	AR
SERAC1 (614725)	3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome (MEGDEL)	AR
SGCE (604149)	DYT11: Myoclonus-dystonia	AD
SLC16A2 (300095)	Allan-Herndon-Dudley syndrome (monocarboxylate transporter-8 (MCT8) deficiency)	XD
SLC19A3 (606152)		AR

	Thiamine transporter deficiency (formerly Biotin responsive basal ganglia disorder)	
SLC20A1 (137570)	Familial idiopathic basal ganglia calcification	AD
SLC2A1 (138140)	DYT9/18: Paroxysmal choreoathetosis with episodic ataxia and spasticity/GLUT1 deficiency syndrome-1	AD
SLC30A10 (611146)	Dystonia with brain manganese accumulation	AR
SLC6A19 (608893)	Hartnup disease	AR
SLC6A3 (126455)	Infantile parkinsonism-dystonia (Dopamine transporter deficiency)	AR
SPG11 (610844)	Spastic paraplegia type 11	AR
SPG7 (602783)	Spastic paraplegia type 7	AR
SPR (182125)	Sepiaterine reductase deficiency	AR
SUCLA2 (603921)	Mitochondrial DNA depletion syndrome 5	AR
SUCLG1 (611224)	Mitochondrial DNA depletion syndrome 9	AR
SURF1 (185620)	Mitochondrial complex IV deficiency/Leigh syndrome	AR
TACO1 (612958)	Mitochondrial complex IV deficiency/Leigh syndrome	AR
TAF1 (313650)	DYT3: X-linked dystonia-parkinsonism	XR
TH (191290)	Tyrosine hydroxylase deficiency	AR
THAP1 (609520)	DYT6: Adolescent onset torsion dystonia, mixed type	AD
TIMM8A (300356)	Mohr-Tranebjaerg syndrome (Dystonia deafness syndrome)	XR
TOR1A (605204)		AD

	DYT1: Early-onset generalized isolated dystonia (PTD)	
TREX1 (606609)	Aicardi-Goutieres syndrome 1	AR, AD
TUBB4A (602662)	DYT4: Whispering dystonia	AD
VPS13A (605978)	Choreoacanthocytosis	AR
Wdr45 (300894)	Neurodegeneration with brain iron accumulation (NBIA) 5	XD

Note: This list of genes excludes several dystonia-associated genes, for example the spinocerebellar ataxias genes, because gene panel analysis cannot detect repeat expansions, whole exon duplications or deletions. Furthermore, the *ATM* gene (ataxia telangiectasia) was omitted from the list, after much debate within our multidisciplinary team, given the fact that carriers of *ATM* have an increased risk for breast cancer. Finally, because a limited amount of genes that could be included in the panel, the *HPRT1* gene (Lesch-Nyhan syndrome) was left out, because Lesch-Nyhan syndrome can be easily diagnosed by testing uric acid in plasma, saving space in the panel for other genes. However, both *ATM* and *HPRT1* will be included in the updated version of the dystonia gene panel that currently is being implemented in our centre.

Abbreviations: OMIM, Online Mendelian Inheritance in Man (www.omim.org); AR, autosomal recessive; AD, autosomal dominant; XR, X-linked recessive; XD, X-linked dominant.

Table 1. Patients characteristics

Age of onset of dystonia	**Number (%)**	**Age on first visit (SD)**	**Academic referrals***
0-2 years	18 (29.5)	17.5 (±14.8)	6 (33.3)
3-12 years	17 (27.9)	18.9 (±13.6)	4 (23.5)
13-20 years	9 (14.7)	34.9 (±19.2)	0 (0.0)
21-40 years	8 (13.1)	46.4 (±12.2)	2 (25.0)
>40 years	9 (14.7)	63.3 (±8.8)	4 (44.4)
Overall	61 (100)	31.0 (±21.8)	16 (26.2%)

*Academic referrals: these patients were referred to us from other tertiary centres

Abbreviation: SD, standard deviation.

Table 2. Identified Genetic Causes

Case no.	Child (<19y) or adult	Identified gene	Mode of inheritance	Mutation	Yield	Diagnosed by experts (control group)
1	child	*PRKRA (DYT16)*	AR	c.558G>T p.(Glu186Asp)	Suggestive*	1 of 2
6	adult	MECP2	XD	c.379C>A p.(Pro127Thr)	Solved	1 of 2
16	adult	GCDH#	AR	c.482G>A p.(Arg161Gln) and c.1262C>T p.(Ala421Val) §	Solved	0 of 2
17	Child	PRRT2	AD	c.649dupC	Solved	2 of 2
19	child	TH#	AR	c.1394C>G p.(Ser465Cys)	Suggestive*	0 of 2

27	child	MECP2	XD	c.1178C>T p. (Pro393Leu)	Suggestive*	1 of 2
32	adult	PRRT2	AD	c.649dupC	Solved	2 of 2
37	adult	PRRT2	AD	c.649dupC	Solved	2 of 2
50	Adult	NPC1#	AR	c.2474A>G p.(Tyr825Cys) and c.3019C>G p.(Pro1007Ala) §	Solved	0 of 2

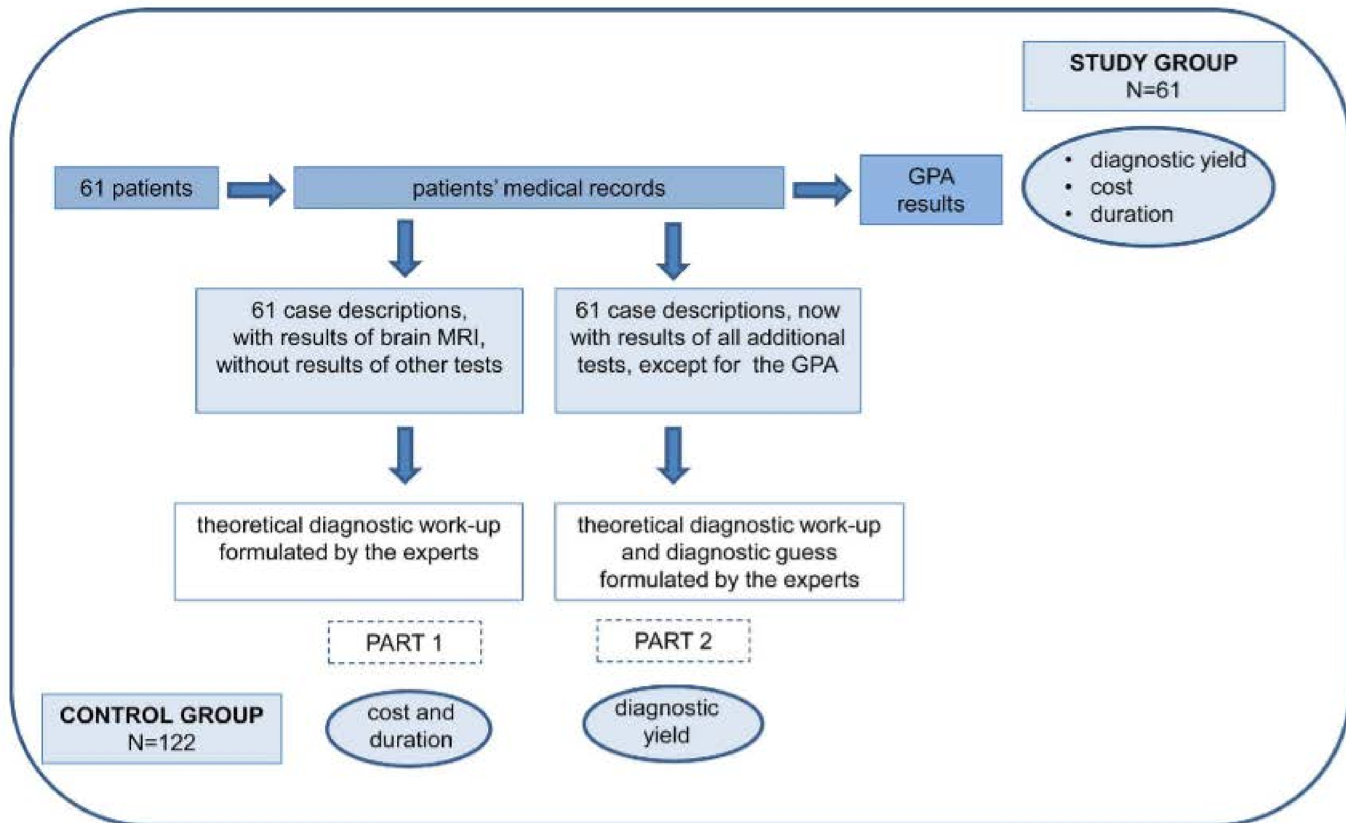
Notes: Identified genetic causes: causal genes found in 9/61 patients and in 9/122 of the theoretical cases. Patients 17, 32 and 37 are not related.

* Suggestive yield means that our multidisciplinary team of clinicians and laboratory staff considered the results of genetic testing highly suggestive for a diagnosis, which often was afterwards confirmed by additional biochemical testing and/or molecular investigations of family members. With regard to case 1: the heterozygous mutation was considered causative based on reports of patients with heterozygous mutations in the *PRKRA* gene with a very similar phenotype[14], and the patient had an excellent response on pallidal stimulation which is in favor of an isolated dystonia such as DYT16. Concerning case 19: it was taken into account that over 10% of *TH* mutations can be found in the promotor region of the *TH* gene[15], these mutations will not be detected in a gene panel strategy. Therefore, in this patient a lumbar puncture was performed showing low homovanillic acid in CSF, confirming the diagnosis of TH deficiency.

# indicates a treatable inborn error of metabolism

§ indicates compound heterozygosity

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XD, X-linked dominant.


STUDY GROUP
N=61
61 patients
patients' medical records
GPA results
diagnostic yield
cost
duration
61 case descriptions, with results of brain MRI, without results of other tests
61 case descriptions, now with results of all additional tests, except for the GPA
theoretical diagnostic work-up formulated by the experts
theoretical diagnostic work-up and diagnostic guess formulated by the experts
PART 1
PART 2
CONTROL GROUP
N=122
cost and duration
diagnostic yield