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ORIGINAL ARTICLE

Effectiveness of clinical exome sequencing in adult patients with difficult-to-diagnose neurological disorders

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Abstract

Objectives: Clinical diagnostics in adults with hereditary neurological diseases is complicated by clinical and genetic heterogeneity, as well as lifestyle effects. Here, we evaluate the effectiveness of exome sequencing and clinical costs in our difficult-to-diagnose adult patient cohort. Additionally, we expand the phenotypic and genetic spectrum of hereditary neurological disorders in Finland.

Methods: We performed clinical exome sequencing (CES) to 100 adult patients from Finland with neurological symptoms of suspected genetic cause. The patients were classified as myopathy ($n = 57$), peripheral neuropathy ($n = 16$), ataxia ($n = 15$), spastic paraplegia ($n = 4$), Parkinsonism ($n = 3$), and mixed ($n = 5$). In addition, we gathered the costs of prior diagnostic work-up to retrospectively assess the cost-effectiveness of CES as a first-line diagnostic tool.

Results: The overall diagnostic yield of CES was 27%. Pathogenic variants were found for 14 patients (in genes *ANO5*, *CHCHD10*, *CLCN1*, *DES*, *DOK7*, *FKBP14*, *POLG*, *PYROXD1*, *SCN4A*, *TUBB3*, and *TTN*) and likely pathogenic previously undescribed variants for 13 patients (in genes *ABCD1*, *AFG3L2*, *ATL1*, *CACNA1A*, *COL6A1*, *DYSF*, *IRF2BPL*, *KCNA1*, *MT-ATP6*, *SAMD9L*, *SGCB*, and *TPM2*). Age of onset below 40 years increased the probability of finding a genetic cause. Our cost evaluation of prior

diagnostic work-up suggested that early CES would be cost-effective in this patient group, in which diagnostic costs increase linearly with prolonged investigations.
Conclusions: Based on our results, CES is a cost-effective, powerful first-line diagnostic tool in establishing the molecular diagnosis in adult neurological patients with variable symptoms. Importantly, CES can markedly shorten the diagnostic odysseys of about one third of patients.

KEYWORDS
clinical exome sequencing, cost analysis, diagnostics, neurological disease

1 | INTRODUCTION

In medicine, neurological and neuromuscular disorders are among the most challenging to diagnose due to the complexities of the nervous system. Sometimes the diagnostic process may take several years and involve a wide range of diagnostic tests. The etiologies of such difficult-to-diagnose disorders may include genetic or acquired factors or both.

Genetic testing has been used increasingly in neurological diagnostics following the developments in high-throughput sequencing technologies.[1] A suspected genetic cause can be investigated by candidate gene sequencing if the symptoms point to a specific single-gene disorder, or by gene panel sequencing in conditions with locus heterogeneity. Gene panel testing has considerably benefitted diagnostics of genetically heterogeneous diseases such as hereditary axonal neuropathies,[2–4] spastic paraplegias,[5] epilepsies,[6] and limb-girdle muscular dystrophies (LGMD).[7]

Clinical exome sequencing (CES) interrogates all disease-associated coding regions of a patient's genome simultaneously and offers an advantage when the clinical picture does not point to the underlying cause. The utility of CES for finding a genetic cause of disease is reasonably good when the pre-test probability of a genetic etiology is high, for example, when the disorder is early onset, or when the family history is positive.[8–11] However, only a few studies have directly and prospectively addressed the likelihood of finding causative genetic variants by CES in adult patients with complex neurologic diseases.[12,13]

The value of an accurate genetic diagnosis is significant. It enables genetic counseling, eliminates the need for further invasive or expensive diagnostic testing, and may influence treatment decisions. For example, hereditary neuropathy is sometimes misdiagnosed as chronic inflammatory demyelinating polyradiculoneuropathy, and treated unnecessarily with immunosuppressive drugs.[14] Patients carrying specific gene defects may also become candidates for new metabolic treatment options such as serine supplementation in hereditary sensory neuropathies[15,16] and niacin in mitochondrial myopathies.[17]

Here, we aimed to assess the effectiveness and cost savings of CES in 100 difficult-to-diagnose adult patients who presented at neurological outpatient clinics in Finland and were suspected of having a genetic disease. Our findings indicate early CES to be cost-effective and shorten the diagnostic odyssey of adult neurological patients.

2 | SUBJECTS AND METHODS

2.1 | Subject recruitment

We recruited 100 subjects, index patients in their families, for CES at adult neurological outpatient clinics in Finland during 2016–2019. The inclusion criteria were as follows:

1. Age ≥16 years at date of testing.
2. Presence of long-standing (≥1 year) neurological symptom(s) of unknown etiology with possible genetic cause, the identification of which would benefit the clinical assessment.
3. No previous molecular diagnosis.
4. No clinical indication for a known common genetic etiology (causing ≥10% of similar cases in Finnish population), which is typically investigated by a single-gene test (eg, *PMP22* duplication in demyelinating peripheral polyneuropathy).

Other diagnostic and therapeutic procedures were carried out according to standard methods as directed by the treating clinician. All participating individuals gave written informed consent. The study was approved by the ethics committee of HUS Helsinki University Hospital. Blood samples were collected for isolation of genomic DNA by standard methods.

2.2 | Clinical exome sequencing

CES was performed at the Finnish Institute of Molecular Medicine (FIMM) with NimbleGen SeqCap EZ Exome as described in.[18] Reads were then aligned to the GRCh37 reference genome.

2.3 | Variant filtering

CES data were filtered for variants in known clinically relevant genes that fulfilled all of the following criteria:

TABLE 1 Demographics of the studied patient groups

	ALL ($n = 100$)		COST ANALYSIS ($n = 60$)	
Patient group	**Median**	**Range**	**Median**	**Range**
Age of onset (years)	37	0–71	38	0–71
Age at first clinical visit (years)	43	0–77	45	16–74
Years from first clinical visit to CES	3	1–48	1	0–7
Age at CES (years)	49	17–82	47	17–74
Sex	51% Male; 49% Female		57% Male; 43% Female	
Origin	93% Finnish, 7% Others		90% Finnish; 10% Others	
CES diagnostic rate	27%		28%	

1. Variants were in genes with previous disease association (OMIM or ClinVar).
2. Variants were predicted to alter protein sequence (missense, nonsense, frameshift, splice site, and short indel variants).
3. Variants had a population frequency of less than 1.0E-03 in gnomADv2.1 variant database and in the Finnish sub-population of the same database.
4. Variants were present in less than 1% of an in-house CES variant database of 429 samples.

Variants were assessed based on ACMG standards[19] as pathogenic (P) if they had been previously reported in a similar phenotype, or likely pathogenic (LP) if they had not been previously reported but were in a known disease gene, which matched the patient's phenotype and inheritance mode. In addition, we listed as variants of unknown significance (VUS) rare heterozygous variants in dominant disease genes and rare homozygous or compound heterozygous variants in recessive disease genes, if they at least partially overlapped with the patient's phenotype.

2.4 | Sanger sequencing and segregation

We used Sanger sequencing to confirm identified variants and to investigate their segregation if samples from family members were available. Sequencing primer sequences and PCR conditions are available on request.

2.5 | Cost analysis

For calculating the costs of traditional diagnostic routine, we studied the records of 60 patients who had had their first clinical visit after year 2010. Patients who had been examined before 2010 were excluded from the cost analysis because (1) full data on costs were not available before that year and (2) the development of diagnostic procedures reduced the comparability of procedures done prior to 2010. Clinical costs that had occurred before the end of 2018 were included. The specific prices were gathered from Helsinki University Hospital and healthcare rates 2017 and 2019, HUSLAB-service rates 2017, Finnish Institute for Health and Welfare (THL) unit rates 2011, Nordlab rates 2017, Tykslab (Turku University hospital) rates 2019 and directly from other service providers. All prices were discounted to year 2018 prices.

2.6 | Statistics

For statistical analysis, patients were divided by CES findings, age categories, or phenotypes. Student's t test (unpaired, two-tailed, GraphPad Prism) was used when comparing the effect of age of onset on CES findings, and Fisher's exact test (two-sided, GraphPad Prism) when comparing patients grouped by disease category or age of onset. Linear regression of cost accumulation was analyzed with GraphPad Prism. Comparisons with $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Study cohort

Demographics of the entire cohort of 100 patients and the 60 patients included in the cost analysis are presented in Table 1. All patients were evaluated in adulthood, although seventeen patients had a pediatric (before the age of 16 years) disease onset. The age of onset ranged from 0 to 71 (median 37) years, and the time from first clinic visit to CES was between 0 and 48 (median 4) years, highlighting the variability of patients and their diagnostic journeys in this cohort. No patients were from consanguineous families and none of the index patients had family members with genetic diagnosis upon testing. We classified the symptomatology of the recruited individuals based on clinical synopses: myopathy ($n = 57$), peripheral neuropathy ($n = 16$), ataxia ($n = 15$), spastic paraplegia ($n = 4$), Parkinsonism ($n = 3$), and mixed ($n = 5$). The mixed group consisted of more complex phenotypes with features from multiple disease categories.

TABLE 2 Pathogenic and likely pathogenic variants identified in this study

Patient	Origin	Disease	Diagnosis synopsis	AoO (year)	Gene	Genomic
HT68	FIN	Myo	Myopathy, scoliosis, hearing impairment	37	FKBP14	7:30058726
HT79	FIN	Myo	Progressive distal muscular dystrophy, no cardiac involvement, CK 353–365	35	DES	2:220285661
HT86	FIN	Myo	Progressive muscular dystrophy, CK 3770	51	ANO5	11:22296151 & 11:22296185
HT87	FIN	Myo	Muscle dystrophy and cardiomyopathy, CK 502	48	TTN	2:179391925–179391935
HT103	FIN	PNP	Spinal muscular atrophy (SMAJ)	42	CHCHD10	22:24109625
HT137	FIN	ATX	Gait and speech ataxia, sensory neuropathy (MIRAS)	29	POLG	15:89866657
HT142	FIN	Myo	Frequent muscle cramps and mild weakness, CK 158	6	SCN4A	17:62022974
HT145	FIN	Myo	Congenital myasthenic syndrome, ventilatory help	0	DOK7	4:3495085 & 4:3495215
HT165	FIN	Myo	Musculur dystrophy, CK 4030–5825	40	ANO5	11:22296151
HT161	FIN	Mixed	Neuropathy, Tonsillar ectopia, extraocular muscle fibrosis	35	TUBB3	16:90002108
HT89	FIN	Myo	Progressive proximal muscular dystrophy, CK 262–4241	34	TTN	2:179391925–179391935
HT166	FIN	Myo	Muscle cramps, rigidity	8	CLCN1	7:143048771
HT64	FIN	Myo	Progressive proximal muscle dystrophy	10	PYROXD1	12:21605064
HT117	FIN	Myo	Progressive proximal muscle dystrophy	49	PYROXD1	12:21605064 & 12:21615741
HT59	FIN	SPAST	Spasticity and lower limb weakness	3	ATL1	14:51094951
HT61	FIN	Myo	Progressive muscular dystrophy, CK 6140	15	SGCB	4:52895932 & 4:52904439
HT81	FIN	SPAST	Spasticity, lower limb weakness and neuropathy	27	ABCD1	X:153008694
HT83	AFGAN	Myo	Proximal muscular dystrophy, mild left ventricle dysfunction, CK 989–3874	20	DYSF	2:71883301
HT85	FIN	SPAST	Spasticity and lower limb weakness	28	ABCD1	X:152990951
HT73	FIN	ATX	Ataxia and cerebellar atrophy	63	CACNA1A	19:13428133
HT72	FIN	Myo	Muscle weakness with cotractures, CK 247	0	TPM2	9:35685287
HT76	FIN	Myo	Congenital progressive myopathy, CK 86	0	COL6A1	21:47410172
HT101	FIN	ATX	Ataxia, dysarthria, lower limb spasticity	21	AFG3L2	18:12337348 & 18:12353120
HT131	FIN	Mixed	Neuropathy and cerebellar ataxia, IgA nephropathy	25	MT-ATP6	M:9154
HT102	FIN	ATX	Ataxia, cerebellar atrophy	40	SAMD9L	7:92762485
HT77	FIN	Mixed	Ataxia, dysarthria, recurrent psychosis, cognitive impairment	28	IRF2BPL	14:77493551
HT160	FIN	Myo	Calf hypertrophy	58	KCNA1	12:5020689

Note: Nucleotide and amino-acid locations are based on Ensemble Gencode canonical transcripts and proteins.

Abbreviations: AoO, age of onset; ATX, ataxia; Myo, myopathy; PNP, polyneuropathy; SPAST, spasticity.

[†]Segregation studied by Sanger sequencing.

Nucleotide	Aminoacid	GnomADv2.1 (all)	Category	Reference
c.362_363insC	hom p. Glu122ArgfsTer7	–	Pathogenic	25
c.1009G>C	het p. Ala337Pro	–	Pathogenic	23
c.2272C>T & c.2311_2312delCA	comp het p. Arg758Cys & p. Gln771AlafsTer8	6.58E−04 & –	Pathogenic	20
c.80585_80595delinsTGAAAGAAAAA	het p. Glu26862_Trp26865delinsValLysGluLys	–	Pathogenic	30
c.197G>T	het p. Gly66Val	1.76E−05	Pathogenic	21
c.2243G>C	hom p. Trp748Ser	9.90E−04	Pathogenic	26
c.3466G>A	het p. Ala1156Thr	5.32E−05	Pathogenic	28
c.1378dupC & c.1508dupC	comp het p. Gln460ProfsTer59 & p. Pro504SerfsTer15	5.42E−5 & 3.29E−4	Pathogenic	24
c.2272C>T	hom p. Arg758Cys	6.58E−04	Pathogenic	20
c.1249G>A	het p. Asp417Asn	–	Pathogenic	29
c.80585_80595delinsTGAAAGAAAAA	het p. Glu26862_Trp26865delinsValLysGluLys	–	Pathogenic	30
c.2680C>T	het p. Arg894Ter	0.003182	Pathogenic	22
c.464A > G	hom p. Asn155Ser†	4.55E−05	Pathogenic	18,27
c.464A>G & c.1061A>G	comp het p. Asn155Ser & p. Tyr354Cys †	4.55E−5 & 1.42E−5	Pathogenic	18,27
c.1322T>C	het p. Ile441Thr †	–	Likely pathogenic	This publication
c.341C>T & c.−10−22del32	comp het p. Ser114Phe & c.−10−22del32	2.72E−4 & –	Likely pathogenic	This publication
c.1885G>T	het p. Asp629Tyr	–	Likely pathogenic	This publication
c.4636A>C	hom p. Thr1546Pro	–	Likely pathogenic	This publication
c.230G>A	hemi p. Trp77Ter †	–	Likely pathogenic	This publication
c.1348T>C	het p. Ser450Pro	7.17E−06	Likely pathogenic	This publication
c.541_542GA>AG	het p. Glu181Arg †	–	Likely pathogenic	This publication
c.931G>T	het p. Gly311Cys †	–	Likely pathogenic	This publication
c.2167G>A & c.1202C>T	comp het p. Val723Met & p. Pro401Leu †	1.77E−4 & 1.8E−4	Likely pathogenic	This publication
c.628C>T	heteroplasmic p. Gln210Ter	–	Likely pathogenic	This publication
c.2800G>C	het p. Asp934His †	–	Likely pathogenic	This publication
c.584delG	het p. Gly195AlafsTer17	–	Likely pathogenic	This publication
c.145G>C	het p. Glu49Gln†	3.98E−06	Likely pathogenic	This publication

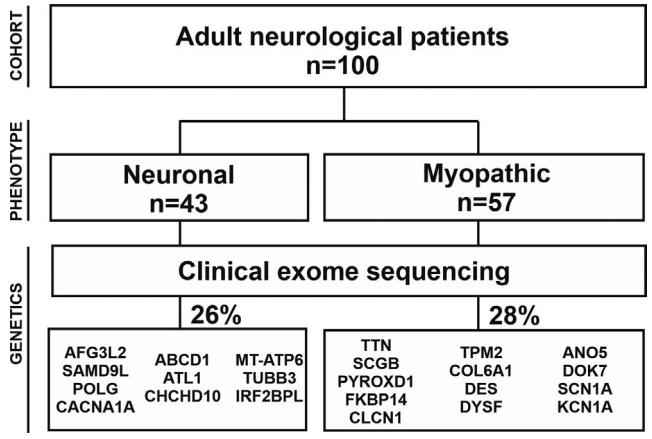

FIGURE 1 Study cohort. One hundred adult patients were recruited to the study. The patients were phenotypically divided into "Neuronal" (peripheral neuropathy, ataxia, spastic paraplegia, Parkinsonism, or mixed phenotypes) and "Myopathic" groups. Percentage of solved cases are indicated for each group, as well as the identified genes with pathogenic or likely pathogenic variants

3.2 | Diagnostic rate of CES

The filtering and assessment of CES data yielded pathogenic variants for 14 patients (14%) in 11 genes (*ANO5*,[20] *CHCHD10*,[21] *CLCN1*,[22] *DES*,[23] *DOK7*,[24] *FKBP14*,[25] *POLG*,[26] *PYROXD1*,[18,27] *SCN4A*,[28] *TUBB3*,[29] and *TTN*[30]) and LP variants for 13 patients (13%) in 12 genes (*ABCD1*, *AFG3L2*, *ATL1*, *CACNA1A*, *COL6A1*, *DYSF*, *IRF2BPL*, *KCNA1*, *MT-ATP6*, *SAMD9L*, *SGCB*, and *TPM2*) (Table 2, Figure 1). Thus, the overall diagnostic rate of cases solved by CES was 27%. Of the solved cases, 14 (52%) variants were autosomal dominant, 10 (37%) autosomal recessive, two (7%) X-linked recessive and one (3%) mitochondrial. Two variants could be confirmed to have occurred *de novo*. A further 18 (18%) individuals had at least one VUS (Table S1).

For the solved cases, the age of symptom onset (median 29 years; range 0–63 years) was significantly lower than in the patients who did not receive a genetic diagnosis by CES (median 40 years; range 0–71 years) (*t* test, $p = .034$) (Figure 2A). Age of onset below 40 years markedly increased the diagnostic yield (Figure 2B). We found no effect of gender or time between the first presentation to a neurologist and CES on the diagnostic rate.

To investigate whether the diagnostic success by CES depended on the phenotype, we divided the cohort into muscle-originating (myopathic) and neural groups (neuropathy, ataxia, spastic paraplegia, Parkinsonism, and mixed). In the myopathy group, 16/57 (28%) of the patients received a genetic diagnosis in comparison with 11/43 patients (26%) in the neural group (Fisher's exact, $p = 0.82$) (Figure 2C). In conclusion, the age of onset but not the phenotype had an influence on the outcome of CES in this study.

3.3 | Pathogenic variants with atypical phenotypes

Two Finnish founder variants, *CHCHD10* p. Gly66Val and *TTN* Finnmajor, were identified by CES in patients with atypical disease

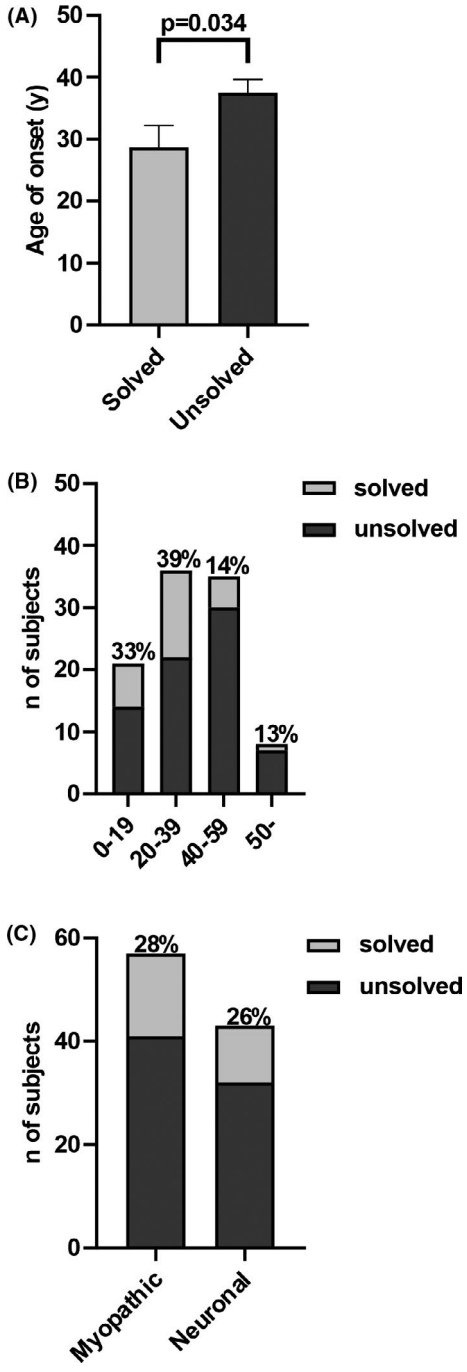

FIGURE 2 Diagnostic determinants. A. Diagnostic yield by age of onset. Average age of onset of subjects with a likely pathogenic or pathogenic variant (solved, $n = 27$) and subjects without findings (unsolved, $n = 63$). Error bars are standard error of the mean (SEM). Student's t test. B. Diagnostic yield by age of onset groups. Subjects were categorized into groups based on patient-reported age at symptom onset. Solved cases are shown with light gray and unsolved with dark gray. Percentage of solved cases per age group is indicated. C. Diagnostic yield by phenotypes. Subjects were categorized into groups based on myopathic or neuronal phenotype. Solved cases are shown with light gray and unsolved with dark gray. Percentage of solved cases per phenotype group is indicated

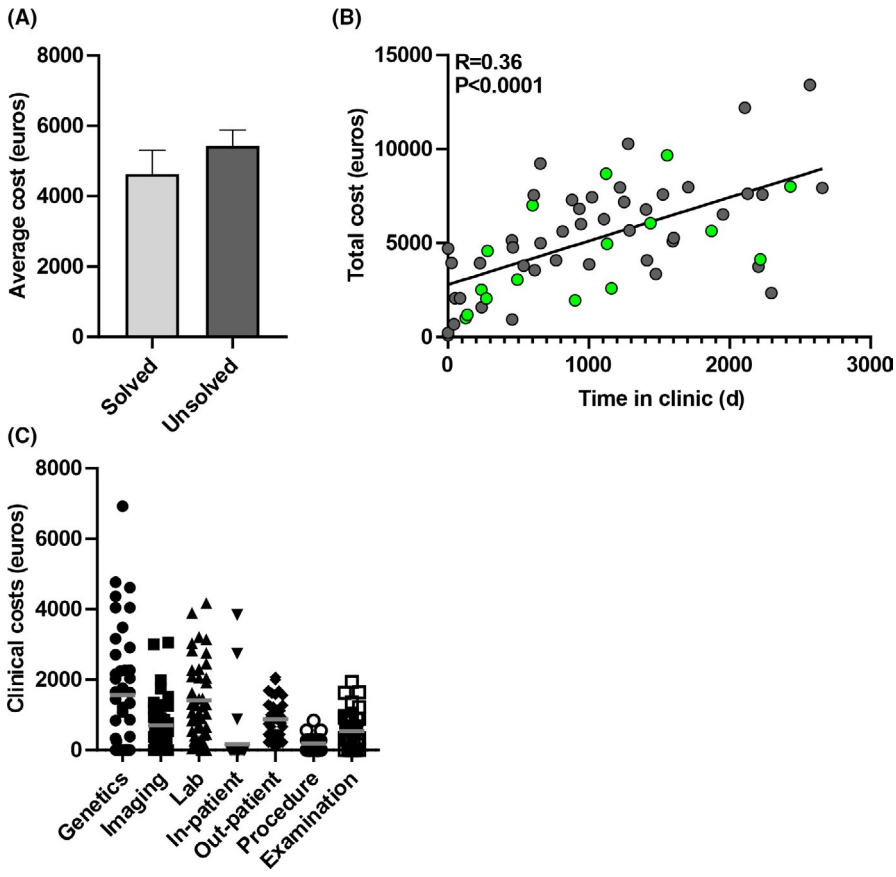

FIGURE 3 Diagnostic costs. A. Average costs of all diagnostic procedures for subjects with a likely pathogenic or pathogenic variant (solved, $n = 17$) and subjects without findings (unsolved, $n = 43$). Costs were calculated for subjects with first clinical visit after 2010 ($n = 60$). Error bars are SEM. Student's t test, no significant difference between groups. B. For the cohort of subjects presenting after 2010 ($n = 60$), total cost (euros) are plotted against the time (days) since the first presentation to the neurologist. Cases solved by CES in this study are highlighted with green ($n = 17$). Linear regression. C. Costs of different diagnostic procedures for subjects with first clinical visit after 2010 ($n = 60$). Each point refers to specific diagnostic costs of an individual. Gray line indicates the mean

presentations. Patient with the *CHCHD10* variant was clinically suspected of having Charcot-Marie-Tooth neuropathy, and only following the genetic finding could be assigned the diagnoses of spinal muscular atrophy Jokela type (SMAJ, MIM#615048).[21,31] One patient (HT89) with heterozygous *TTN* Finn-major variant had a severe myopathy with inflammatory features; the severity of symptoms is not common in a dominant titinopathy (MIM#600334).[30] In comparison, another patient (HT87) with the heterozygous Finn-major variant had a more typical representation of disease with muscular dystrophy, in addition to cardiomyopathy. Surprisingly, also a patient with mitochondrial recessive ataxia syndrome (MIRAS, MIM#607459)[26] was detected in the cohort although the patient's family history suggested a dominantly inherited disease.

We have previously reported two male patients of this cohort with *PYROXD1* variants, who were either homozygous or heterozygous for p. Asn155Ser. The same variant was originally reported to cause early-onset myofibrillar myopathy (MIM#617258),[27] whereas the patients in our cohort had adult-onset limb-girdle muscular dystrophy (LGMD), thus revealing a new phenotype associated with *PYROXD1*.[18]

3.4 | New likely pathogenic variants in known disease genes

Brief description of the LP variants for 13 patients (13%) in 12 genes (*ABCD1*, *AFG3L2*, *ATL1*, *CACNA1A*, *COL6A1*, *DYSF*, *IRF2BPL*, *KCNA1*, *MT-ATP6*, *SAMD9L*, *SGCB*, and *TPM2*) is in Table 2, and detailed description can be found in Supplementary File 1. DNA samples of family members were investigated for variant segregation when available (please see pedigree information in Figure S1).

3.5 | Variants of unknown significance

We additionally listed 23 variants of unknown significance (VUS) for 18 patients, which were of interest but currently lack sufficient evidence for pathogenicity. These were in genes *ATL3*, *CAPN3*, *CHAT*, *CHD1*, *CLCN1*, *COL6A2*, *COL6A3*, *COL9A3*, *CPT2*, *FBLN5*, *GALC*, *MEGF10*, *MYPN*, *NF2*, *PCYT2*, *PEIZO2*, *RYR1*, *SCN91*, *TNNT3*, and *TWNK* (Table S1).

3.6 | Cost analysis

We calculated retrospectively the diagnostic costs prior to CES for the group of patients who had their first presentation to a neurologist after year 2010 ($n = 60$). In this subgroup, the diagnostic rate of CES was 28% (17 P/LP out of 60). Those who received a diagnosis by CES had an average of 2.6 years (range 0.1 to 6.7 years) follow-up time since the first presentation to clinic. We included costs from the following categories: genetics, imaging, laboratory assays, examinations, procedures as well as patient care in outpatient and inpatient clinic. Total average costs per patient were €5200 (range €90

to €13 400). The diagnostic costs increased linearly with the duration of time from first clinical visit to CES (Linear regression, $r = 0.36$, $p < .0001$) (Figure 3B). There was no significant difference in total costs prior to CES between patients who received a genetic diagnosis and those who did not (*t* test, $p = 0.34$) (Figure 3A). Figure 3C shows the distribution of costs between categories per patient, with the largest proportion, 28%, of costs arising from genetic testing such as single-gene Sanger sequencing or gene panels. Costs in the other categories were distributed as follows: laboratory assays 26%, imaging 14%, examinations 10%, in- and outpatient care 19%, and procedures 4%.

Next, we calculated the cost savings of CES as a first-line diagnostic test. Prior to CES, total diagnostic costs in this subgroup of 60 patients were €312,000, of which €87,000 were genetic testing costs, leaving €225,000 for other than genetic costs. Using the obtained 28% success rate of CES, hypothetical first-line CES would have removed all or most occurred diagnostic costs for the 28% of patients who received a genetic diagnoses. In addition, the costs that occurred from other genetic tests to any patient would have been saved by using CES as the first test. Hence in this cohort, the savings would have been 28% of the costs other than the genetic testing (€225,000*28%) plus all costs of the prior genetic testing (€87,000), equaling €150,000. Thus, CES would be cost-effective in this cohort if it was priced below €2500 (€150,000/60).

4 | DISCUSSION

Here, we evaluated the effectiveness of CES in a difficult-to-diagnose group of adult patients with neurological diseases. Included were only index patients with a suspected genetic cause, excluding those whose symptoms pointed directly to a specific single-gene defect. Owing to the extensive genetic heterogeneity behind neurological phenotypes in adults, combined with lifestyle and other modifying effects on symptom onset and rate of progression, it may take several years or even decades to determine the exact diagnoses. Remarkably, four of the cases solved by CES in our cohort had a clinical trajectory of more than 35 years from disease onset to molecular diagnosis. CES has become a routine diagnostic tool in many centers, but may not be an obvious choice for this group of adult patients in all public healthcare systems, in particular as reaching a molecular diagnosis rarely leads to a direct treatment option. Our results support the use of CES in first-line diagnostics of adults with suspected genetic neurological diseases. With the success rate of 27% in our cohort, early CES would have markedly shortened the time to diagnosis for about one third of the patients.

Overall the success rate in our study compares to previously published reports, 17.5%–33%, with similar mainly adult cohorts comprising of patients with varied neurological symptoms.[12,32,33] A higher success rate, up to 42%, has been obtained by rigorous patient selection based on clinical data,[13,34] which is not feasible if CES is used as a first-line tool. In addition, higher success rates are reported in cohorts where only specific disease groups are studied, such as LGMD or peripheral neuropathy.[35,36]

In our study, the age of onset below 40 predicted a higher probability for finding the genetic cause by CES. A similar trend of earlier disease onset predicting a molecular diagnosis was found in a study of 486 adult patients with highly varied neurological symptoms[32] and in a study comprising of 1000 prenatal to adult patients with mainly nervous system abnormalities.[37] This is in line with a higher success rate of CES in pediatric cohorts, because early-onset diseases are more likely to have a genetic than acquired cause, and have more uniform and recognizable symptoms.[8] Also in adults, a lack of parental samples may complicate segregation studies and confirmation of *de novo* variants,[32] which are common causes in pediatric cohorts.[38] Another complication is that less severe adult-onset disorders may go undiagnosed, leading variants to be erroneously categorized as benign.

In clinical setting, CES can only focus on previously confirmed disease-associated genes and variants. Thus, the patients of our cohort who did not receive a genetic diagnosis may have had variants that are not yet identified as pathogenic, in known or still unknown disease genes, or have intronic or regulatory variants, repeat expansions, or copy number variations that were not detected by CES. Whole-genome sequencing offers solutions to overcome some of the technical shortages of CES,[39] but not for the missing knowledge of pathogenic variants and the associated phenotypes. Copy number variation is possible to detect using next-generation sequencing data,[40] but was not investigated in this study. Polygenic inheritance of some neurological diseases has also been proposed,[41,42] but was not assessed here even though some patients were found to have multiple possible phenotype contributing variants (one P or LP and one VUS: HT77 and HT89; two VUS: HT70, HT153, and HT159). Finally, it is possible that some patients had an acquired cause of neurological symptoms.

Identification of a pathogenic variant directly confirms the genetic diagnoses, whereas LP variants typically require additional work such as segregation analysis in the family, serum measurements, histochemistry, or functional analysis, which may not be available as part of diagnostics. In this cohort for example, a novel *DYSF* (dysferlinopathy) variant was confirmed by dysferlin immunostaining in a muscle biopsy (Figure S2), and *ABCD1* (X-linked adrenoleukodystrophy) diagnosis was set by serum measurement of VLCFAs. Interestingly, we also found a few well-known Finnish founder variants (in genes *CHCHD10* and *TTN*), which should have been excluded from our cohort. However, the phenotypes of these patients were somewhat atypical, highlighting again the complexity of clinical diagnostics in adult patients.

Our results show that an early investment to CES would be cost-effective in this patient group if CES was priced below €2500. Amounting evidence supports the cost-effectiveness of early CES in suspected hereditary diseases.[34,43–45] Analyzing cost-effectiveness based on health outcomes needs further assessment. It should be noted that in our retrospective analysis we made several simplifications of the occurred diagnostic costs. For example, neuroimaging and neurophysiological examinations may

be required for evaluation of prognosis even if genetic diagnosis is reached. Furthermore, LP variant findings required additional research efforts for which costs were not calculated; however, such studies are not needed when the variants become classified as pathogenic. Also, we claimed that first-line CES removes all other genetic costs, but this only applies to sequencing costs. Nevertheless, a surprisingly large proportion of the overall diagnostic costs came from single-gene and panel sequencing, which would be avoided by early CES. Importantly, CES as a first-line test has also other benefits than the effects on costs and diagnostic time. Two patients had received expensive immunologic treatment without benefit, which would not have been administered if the molecular diagnosis had been resolved earlier. Also, some invasive procedures, such as muscle biopsies, could have been avoided. Furthermore, as only index patients were studied here, the genetic finding by CES will directly indicate a molecular diagnosis for their affected family members. Genetic counseling is thus important. In upcoming years, as NGS costs decline, it could also be feasible to use WGS as a first-line diagnostic tool, which would allow the reliable detection of non-exonic variants, large indels, repeat expansions, and copy number variations early in the clinical odyssey.

In summary, our results expand the spectrum of disease variants and their associated neurological phenotypes. We recommend CES in adults with difficult-to-diagnose neurological diseases as an effective first-line diagnostic tool, which can markedly shorten the time from symptom onset to diagnoses and have cost savings. In the future, increased knowledge of human disease variation will enable a rapid diagnoses for even a higher proportion of neurological disease patients.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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