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Next-Generation Genetic Testing in the Diagnostics of Neurological Disease in Southwest Finland in 2010–2021: A Register-Based Study

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ABSTRACT

Neurological disorders are heterogeneous and sometimes challenging to diagnose. Next-generation sequencing (NGS) panels and exome sequencing methods are increasingly advocated as first-tier genetic investigations. In this retrospective, single-centre, register-based study, we investigated the use of NGS-based investigations in the diagnostics of adult neurological disease at Turku University Hospital (TUH) (Turku, Finland) during 2010–2021. We identified patients who underwent any genetic testing to investigate neurologic disease in 2010–2021. NGS gene panel studies and exome investigations were scrutinised further. Data were collected from the TUH electronic medical records. We identified $N = 844$ patients (347 men and 497 women) who fulfilled the initial inclusion criteria. In this group, 331 NGS panels and 99 exome analyses were performed. The median age at the time of the first included genetic test was 45 years (range: 16–96 years). The diagnostic rate was 19% for all NGS-based studies. Amongst different patient groups, the diagnostic yield was highest in developmental and intellectual disorders (39%), second highest in neuromuscular disorders (38%) and lower in epilepsy and ataxia (13% and 10%, respectively). Amongst neurological disorders, the diagnostic yield of genetic testing differs between different patient phenotypes and based on the genetic testing selection. Further studies are needed to determine optimal strategies, with the highest yield and lowest cost, for genetic investigations in neurological disorders.

1 | Introduction

Neurological disorders are clinically and genetically heterogeneous and sometimes challenging to diagnose [1–3]. In some neurological disorders, for example, in certain forms of ataxia, there are no clinical findings or symptoms that would suggest a specific molecular aetiology [2]. Yet a definite molecular diagnosis is of importance for genetic counselling, evaluation of recurrence risk and potential prenatal or preimplantation testing. Exact diagnosis may also enable more accurate prognostic evaluations and is relevant when planning individual treatment options [1–6]. Because of recent rapid evolution in genomic testing methods, the landscape of genetic testing amongst neurological patients has also changed dramatically. Next-generation sequencing (NGS) studies, including both panels and exome sequencing, are increasingly advocated as first-tier genetic investigations [7].

NGS enables fast and relatively economic analysis of large sets of genes, and NGS studies are therefore useful and cost-effective when trying to reach a molecular diagnosis [8–10]. NGS panels are increasingly used because of improving availability and

decreased costs [11, 12]. In addition to panels, clinical exome sequencing (CES) (comprising ~4000–5000 genes), whole exome sequencing (WES) (all 20,000 genes) and whole genome sequencing (WGS) are used in the molecular diagnostics of genetic neurological disorders. The scope and number of investigated genes are the basis for differentiating between CES and WES. In CES, only genes known to be associated with certain diseases or symptoms are investigated, whereas in WES, the whole exome is investigated, even though the raw data are filtered by HPO (Human Phenotype Ontology) terms. In more recent times, CES is less used, and almost all postnatal exome studies are performed as WES. Exome sequencing may allow accurate identification of variants with even greater sensitivity. The cost of exome sequencing is not much higher compared to NGS panels [13], but the most expensive are the situations where multiple testing is needed, for example, when a gene panel turns out negative, and this is then followed by an exome analysis. Gene panels target limited areas of the exome and therefore require less space and resources for data processing and analysis than exome analysis [9]. It is also possible to sequence the whole exome but then analyse only a selection of genes (virtual panels) from these data and later expand the scope of analysis if needed [14].

In WGS, the whole genome is analysed, and therefore, it is possible to detect, for example, single nucleotide variants, small insertions or deletions, copy number variants or even mitochondrial DNA variants if the reading depth is sufficient. However, larger repeat expansions may remain undetected with WGS, if their size is over the reading depth [15].

In the Finnish healthcare system, specialised medical care is mostly provided by tax-funded public services (universal healthcare). This is why the availability of genetic testing is not limited by patient insurance policies, and the use of genetic testing is based on the clinical need. In the region of Southwest Finland, specialist medical care and advanced genetic diagnostics in the field of neurology are provided almost exclusively by Turku University Hospital (TUH) (Turku, Finland). Investigations carried out at TUH thus represent well the use and outcomes of these neurogenetic diagnostic investigations in this geographical area. In this retrospective, single-centre, register-based study, we investigated the use of modern, NGS-based investigations including NGS gene panels, CES and WES in the molecular diagnostics of adult neurological disease and syndromes at TUH during the years 2010–2021.

2 | Patients and Methods

Patients investigated at the adult neurology department of TUH (at least 16 years of age at the time of investigations) who had at least one contact with the departments of neurology and medical genetics at TUH and who underwent genetic investigations between January 1, 2010, and December 31, 2021, were included in the study (Table 1). The investigated patients were identified, and data were collected from the TUH electronic medical records (EMRs) that were available for the whole period. The NGS gene panel investigations as well as CES and WES investigations were scrutinised further. We also recorded the amount of WGS investigations, molecular karyotyping, chromosomal analyses, single nuclear gene analyses and mitochondrial DNA analyses, but these were excluded here from further scrutiny. These are mentioned as 'other tests' in the results. Repeat expansion tests were also excluded. No detailed data are presented.

TABLE 1 | Study inclusion and stratification criteria.

- Patient underwent genetic investigations for a neurological symptom or disorder
- Only NGS studies (gene panels and exome studies) included in further scrutiny
- Investigations ordered by physicians at TUH, between January 1, 2010, and December 31, 2021
- Patient at least 16 years of age at the time of genetic investigations during this period
- Patient needed to have at least one contact with the department of neurology and the department of medical genetics at TUH
- Main clinical problem/cause for diagnostic testing determined based on clinical notes, laboratory referrals and ICD-10 codes
- Detected gene variants sorted according to the ACMG classification

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ICD-10, International Classification of Diseases Version 10; NGS, next-generation sequencing; TUH, Turku University Hospital.

All included tests were requested by either a neurologist or a geneticist at TUH. All different NGS-based analyses were included. Some patients had one or several gene panels, some CES, some WES and very rarely WGS, since it was not yet in routine use. In addition to in-house gene panels, there were different panels from various external service providers (both academic centres and commercial providers). Thus, there were some differences in the content of gene panels and sometimes also in the exome studies. The laboratories that provided the gene panels decided independently which genes were included; these were not selected by the clinicians that ordered the tests.

For the patients who underwent some of the predefined investigations, we collected information about the sex of the patient, age at the time of first genetic testing, age at disease onset (either exact age or descriptive such as early childhood or adulthood, depending on available information in the EMRs) and age at reaching a definite molecular diagnosis. We also collected descriptions of clinical symptoms; causes of testing (clinical symptoms or suspected disease) including ataxia, epilepsies, developmental disorders and intellectual disabilities (DDs/IDs) and neuromuscular disorders; and the available family history. The International Classification of Diseases Version 10 (ICD-10) codes for the neurological diagnoses of the patients were used in addition to other clinical information to confirm the classification of patients according to the main neurological phenotype.

All performed genetic tests for each patient were recorded. The genetic test results of the identified patients were collected from the original sign-out reports. Data on the performed genetic analyses, test dates and age at testing and test results were

collected. The results were sorted in three categories: diagnostic 'positive' findings, 'negative' findings meaning that no variant explaining the symptoms or disease was detected or variants of unknown significance (VUSs). Incidental findings were also collected. The detected variants were listed according to the classification guidelines of the American College of Medical Genetics and Genomics (ACMG) [16]. All information was collected from the sign-out reports meaning that only variants of ACMG Classes 3–5 (VUS, likely pathogenic or pathogenic) are reported. Both likely pathogenic and pathogenic variants were interpreted as 'positive'.

3 | Results

We identified $N = 844$ patients (347 men and 497 women) who fulfilled the initial study inclusion criteria. In this group, 331 NGS panels, 77 clinical exome studies and 22 whole exome analyses were performed. The median age of patients at the time of the first included genetic test was 45 years (range: 16–96 years). Amongst the patients, 181 (46%) had a family history of symptoms or the disease. There were 8 tests (1 NGS gene panel, 6 CES and 1 WES) as duo or trio investigations for individuals representing healthy family members of index patients; these are not included in the results. Only probands (index cases) were accessed. The overall median diagnostic delay counted from the onset of symptoms was 17 years, and the mean age of onset was 27 years. The longest diagnostic delay was observed for DDs (24 years) and the shortest for neuromuscular disorders (14 years). The flowchart of the patient identification process and the composition of performed genetic investigations are shown in Figure 1. The list of all variants detected during this research period is available as a supplement (Supporting Information 1 (available here)).

The use of NGS panels and CES for the molecular diagnostics of genetic neurologic disorders in TUH increased during the study period. The first two NGS gene panel investigations in the adult neurology clinic at TUH were performed in 2012, but already in 2016, there were 69 NGS panel investigations. Similarly, there were two CES investigations in 2013, but the number of CES investigations increased steadily and reached 21 tests per year by the end of 2021. The highest number of WES studies performed was in 2019 ($N = 8$). The changes in the utilisation of different genetic tests over time are shown in Figure 2.

During the study period, 679 (80%) of the patients had an NGS-based test or a single gene test as their first test, and 503 (60%) of the patients had only one test performed. A total of 248 (29%) patients had more than one NGS-based test or single gene test (Supporting Information 2 (available here)).

Overall, the diagnostic rate was approximately 19% (82 out of 422) for all NGS panels and exome studies together. The diagnostic yield for the exome studies was 14% (13 out of 92), whereas in the NGS panels, a molecular diagnosis was reached in 21% (69 out of 330) of the cases. All three testing methods gave a negative (normal) result in approximately 52% of the cases. The proportion of VUS findings was similar in NGS panels and in CES tests (27% vs. 30%). Amongst the other types of genetic investigations (WGS investigations, molecular karyotyping, chromosomal analyses, single nuclear gene analyses and mitochondrial DNA analyses), 49 (32.2%) resulted in a molecular diagnosis, and only 15 (6.1%) resulted in a finding of uncertain significance. Overall, there were 27 (6.4%) reported incidental findings amongst the total of 423 exome and genome tests and NGS panels that were performed (Figure 2). We looked at the number of tests performed to diagnose different types of neurological disorders and their results (Figure 3). The largest such

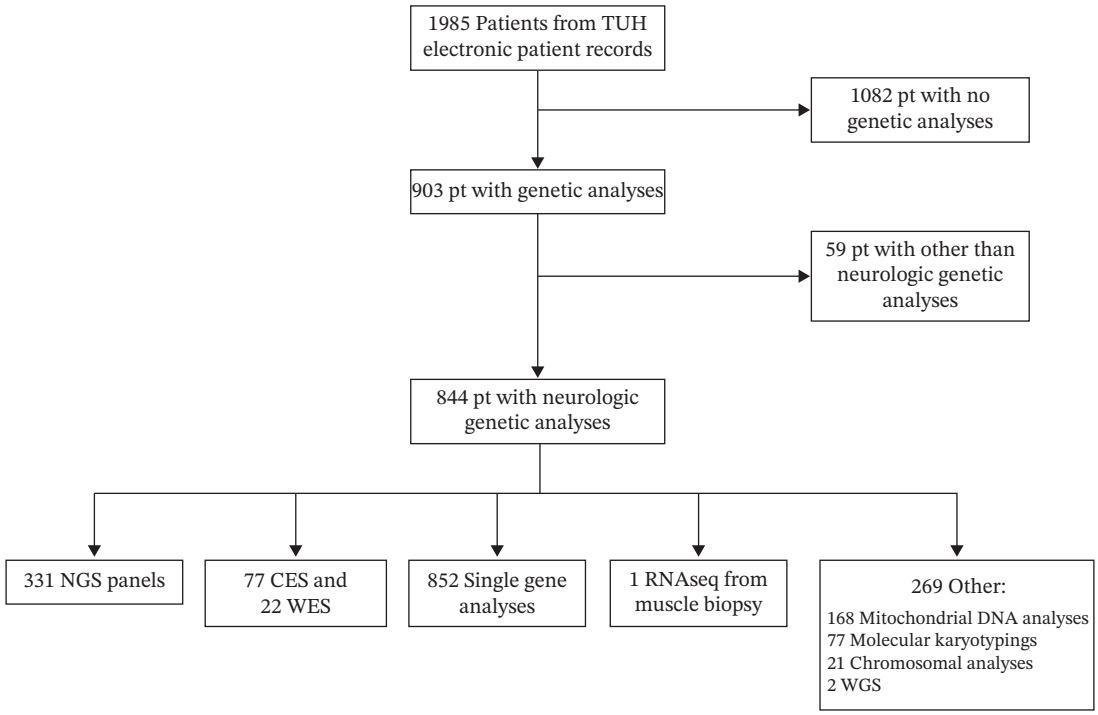


FIGURE 1 | Flowchart of the investigated patients. CES = clinical exome sequencing, NGS = next-generation sequencing, TUH = Turku University Hospital, WES = whole exome sequencing, WGS = whole genome sequencing.

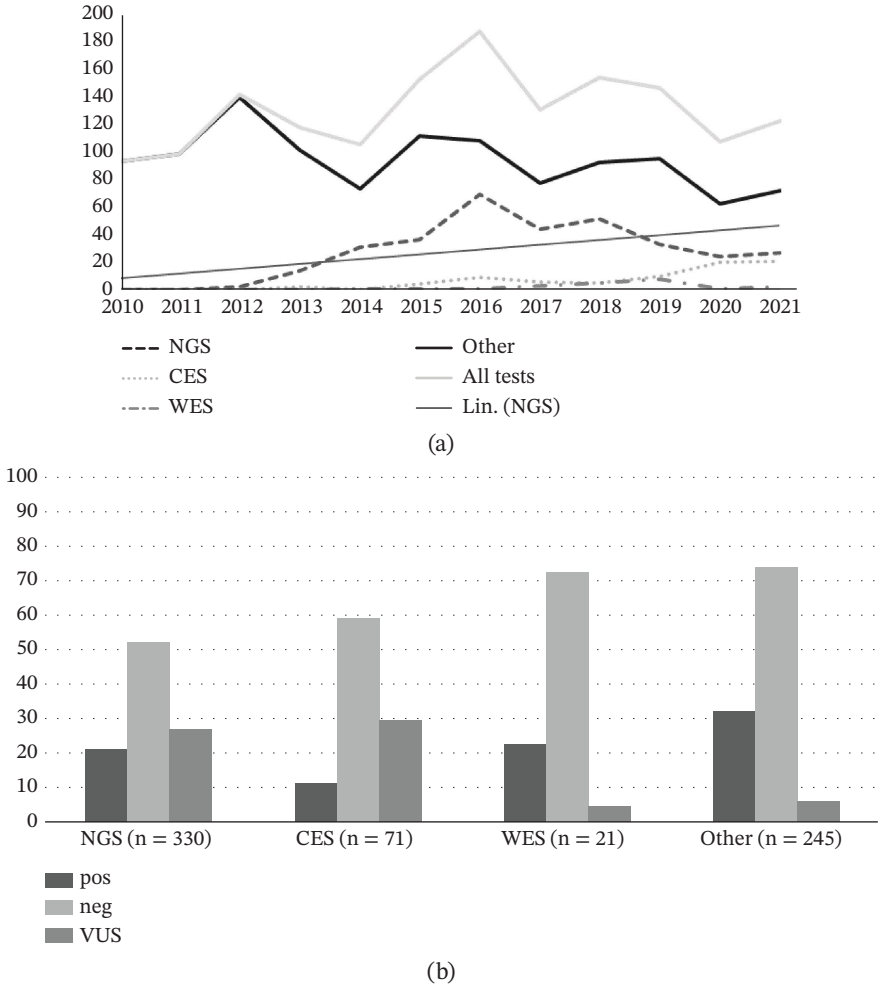


FIGURE 2 | (a) Neurological genetic analyses at Turku University Hospital in 2010–2021. (b) Different types of genetic analyses and their results at Turku University Hospital in 2010–2021. Single gene investigations are not included. CES = clinical exome sequencing, Neg = no relevant variants detected, NGS = next-generation sequencing, Pos = a diagnostic finding, VUS = variant of unknown significance, WES = whole exome sequencing.

groups were various neuromuscular disorders ($N = 91$), ataxia ($N = 84$), epilepsy ($N = 60$) and DDs/IDs ($N = 51$). The diagnostic yields of the NGS-based genetic investigations with comparisons to previously published data are summarised in Table 2.

The investigated neuromuscular disorders included myotonia, muscular dystrophy and myopathy. In this group, 26 out of 69 (38%) gene panel studies resulted in a molecular diagnosis. WES studies provided a diagnostic result for two patients (67%), whereas in CES investigations, a diagnostic finding was reached for just one patient (33%).

Amongst the patients investigated for ataxia, NGS gene panel ($N = 36$) and CES ($N = 12$) investigations resulted in a diagnostic finding in, respectively, 11% and 8.3% of the studies. In the ataxia group, VUS findings were common: NGS panels resulted in VUS findings in 42% and CES studies in 33% of the analyses. WES ($N = 2$) or other types of genetic tests ($N = 34$) did not result in diagnostic findings in patients investigated for ataxia.

In addition to the NGS panels, CES and WES studies that were the focus of this study separate tests for repeat expansion disorders. In total, 144 patients underwent some tests for repeat expansions. No detailed data on these investigations are presented. For 109 ataxia patients, tests for repeat expansions (mostly for spinocerebellar ataxia [SCA] Types 1, 2, 3, 6, 7, 8 and 17) were performed in the first line, with a diagnostic finding in three individuals (3%). For Friedreich ataxia that is rare in the Finnish population [28], there were only 12 performed tests, all with negative results. The repeat expansion variant in *RFC1* described in 2019 [29] was tested at TUH only seven times (including six positive results) between 2010 and 2021. The repeat expansion variant in *FGF14* underlying SCA27b [30] was described only after data collection.

Amongst the patients investigated because of epilepsy, 36 also had DD/ID as a concomitant reason for genetic testing. From all investigated genetic testing techniques, NGS panels ($N = 20$) performed best in the epilepsy group, where two tests (15%) were positive. In CES tests ($N = 7$) performed because of epilepsy with DD/ID, a positive finding was reached in one individual (14%). The number of VUSs in the CES analyses was similar in all epilepsy groups, with or without DD/ID (range: 29%–33%). Amongst the patients investigated with NGS panels ($N = 16$) or ESs ($N = 7$) for DD/ID without epilepsy, CES ($N = 5$) had the best diagnostic yield ($N = 4$, 80%), although the NGS gene panel also provided a diagnostic yield of 25% (four patients).

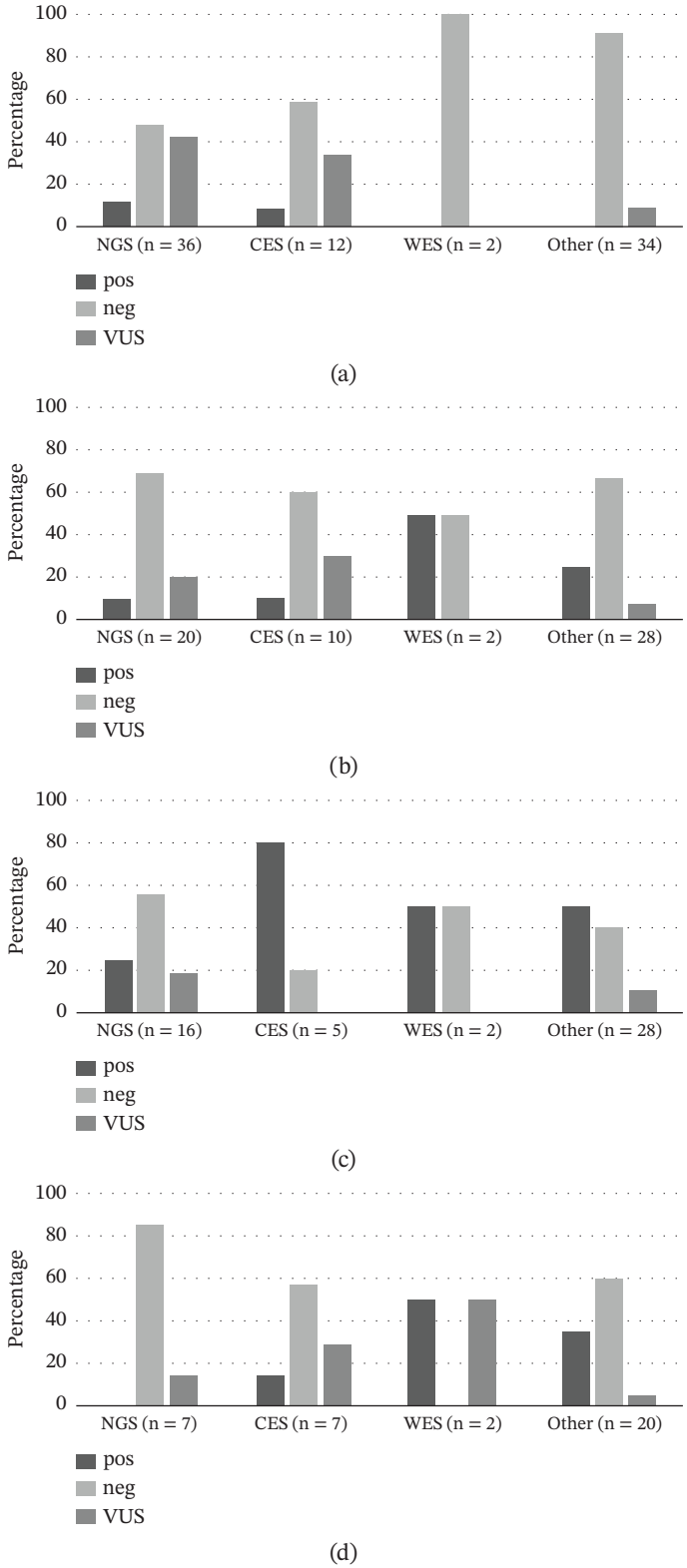
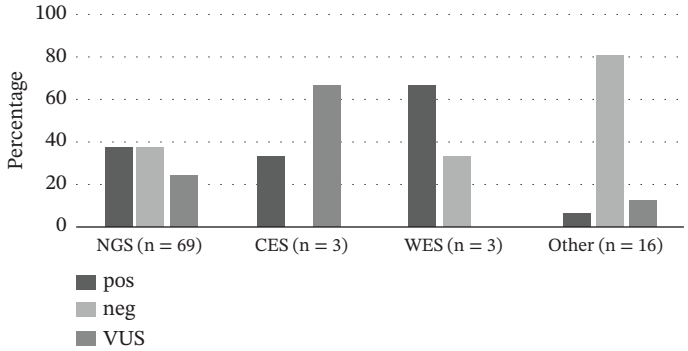


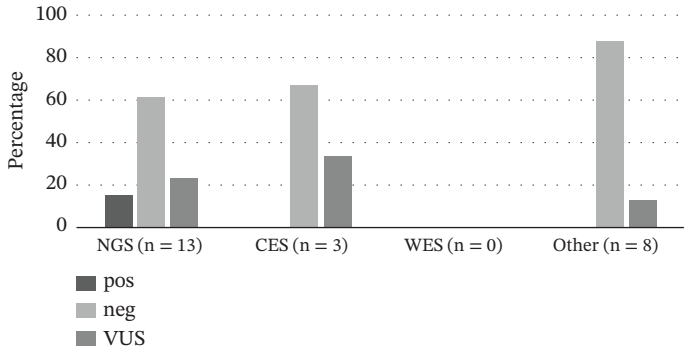
FIGURE 3 | Types of genetic analyses and their results grouped according to the main clinical phenotype. (a) Ataxia, (b) all epilepsies, (c) developmental disorders and intellectual disabilities, (d) epilepsy with intellectual disability and/or developmental disorder, (e) neuromuscular disorders and (f) epilepsy without intellectual disability or developmental disorder. CES = clinical exome sequencing, Neg = no relevant variants detected, NGS = next-generation sequencing, Pos = a diagnostic finding, VUS = variant of unknown significance, WES = whole exome sequencing.

4 | Discussion

In this retrospective, single-centre study, we investigated the utilisation and diagnostic outcomes of genetic testing in the diagnostics of neurological disorders at TUH during 2010–2021. In total, there were 844 patients included in our study, with a median age of 45 (varied between 16 and 96 years). For all NGS panels and exome studies together, the diagnostic yield was



(e)



(f)

FIGURE 3 | (CONTINUED)

19%. Amongst NGS panels, 21% and 23% of WES studies provided a diagnostic result. These are in line with earlier reports [17, 19, 31]. In contrast, the yield of CES was lower as only 11% provided positive results [5, 18]. Between different neurological subgroups, DD/ID (39%) and neuromuscular disorders (39%) provided the highest diagnostic yields for all investigated genetic testing techniques, whereas the yields for epilepsy (13%) and ataxia (10%) were lower.

The focus of this study was to investigate the utilisation and yield of different types of genetic tests rather than the diagnostic pathway of individual patients. However, depending on the phenotype and particularly in the earlier period, the use of single gene or single variant tests and more limited gene panels has been fairly common before advancing to more comprehensive testing such as exome analysis. Reanalysis of exome or genome sequences with more information on pathogenic variants has also resulted in new diagnoses.

Whilst features such as a phenotype typical for a genetic condition and positive family history may increase the likelihood of a diagnostic finding in genetic investigations, we were not able to assess these in this study at the individual level. Based on this retrospective data, we could not assess the precise impact of the genetic diagnosis on clinical care or potential diagnostic delays and their causes. However, even when no precision care was available, a detailed understanding of the disease of the patient has important implications for both clarifying the diagnosis and providing the basis for genetic counselling (e.g., recurrence risk and risk for offspring). In general, challenges in phenotyping and in recognising a condition as potentially genetic in origin are likely important causes of diagnostic delays. In addition, novel pathogenic variants and disease genes are constantly detected, stressing the need of repeated evaluation when a genetic cause is suspected even when initial investigations have been inconclusive.

Data of several other types of genetic testing such as molecular karyotyping, chromosomal analyses, single nuclear gene analyses and mitochondrial DNA analyses were excluded from further scrutiny, since these investigations comprise a large number of disorders but sometimes with a narrower focus. The aim of the present study was to scrutinise the use and diagnostic rate of gene panels and exome studies. Therefore, the focus of this study was on conditions where these investigations have been central. No data on SCA, Friedreich ataxia and *RFC1* studies are presented. For HD, the test for the repeat expansion in *HTT* is typically based on the characteristic clinical phenotype, often with a positive family history of HD. In CMT, a large part of the disease is related to *PMP22* duplications, and in motor neurone disease, to *SOD1* variants and the hexanucleotide repeat expansion in *C9orf72*. In addition, for FTD and ALS, genetic testing is not a uniform part of the diagnostic process. During the investigated period, only two WGS investigations were performed. At the time, the use of WGS was mostly research-based and not part of any routine work-up. This is why WGS investigations were not included in the study. Even though variants in mtDNA may also be detected with WGS, the tissue specificity of variant heteroplasmy levels and the better detection of some common pathogenic variants often require the use of DNA derived from postmitotic tissue such as skeletal muscle instead of white blood cell–derived DNA. The shift from WES towards WGS is approaching. However, whilst it is already a normal routine in some European countries, it is not in Finland. Even though the

TABLE 2 | Diagnostic yields (percentages) from NGS-based genetic investigations in the present study and previous reports.

	Panels		Clinical exome sequencing		Whole exome sequencing		All investigations
	Present data	Previous studies	Present data	Previous studies	Present data	Previous studies	Present data
All neurological diseases	21	23[k]	10	27–53[d,j]	23	27–33[i,l]	19
Ataxia	11	18–55[b,q]	8	CES + WES 13[a,f,n,r]	0	CES + WES 13[a,f,n,r]	10
All epilepsies	10	11–23[c]	10	36[c,h,p]	50	28–38[g,m]	13
Epilepsy without ID/DD	15	n/a	0	CES + WES 9[e]	0	CES + WES 9[e]	13
Epilepsy with ID/DD	0	16–29[o,s]	14	30[n,e]	50	13[s]	13
ID/DD	25	16–21[o]	80	16–45[h,m,n]	50	30[m]	39
Neuromuscular diseases	38	40–60[g]	33	30[n]	67	40[m]	39

Abbreviations: CES, clinical exome sequencing; DD, developmental disability; ID, intellectual disability; NGS, next-generation sequencing; WES = whole exome sequencing.
[a]Pyle et al. [2].
[b]Schuermans et al. [3].
[c]McKnight et al. [4].
[d]Yang et al. [5].
[e]Sheidley et al. [6].
[f]Srivastava et al. [7].
[g]Gonzalez-Quereda et al. [9].
[h]Helbig et al. [10].
[i]Sainio et al. [17].
[j]Yang et al. [18].
[k]Stefanski et al. [19].
[l]Marques Matos et al. [20].
[m]Retterer et al. [21].
[n]Lee et al. [22].
[o]Mellone et al. [23].
[p]Mainali et al. [24].
[q]Németh et al. [25].
[r]Sun et al. [26].
[s]Snoeijen-Schouwenaars et al. [27].

genetics laboratory at TUH is amongst the most advanced in Finland, WGS is not yet in clinical routine use.

Due to the structure of the Finnish public healthcare system, these data represent quite well the whole population. The genetic investigations were performed in the publicly funded healthcare system, ensuring equal access to all people resident in the area. This area of Finland does not include geographically remote or poorly connected areas. The Finnish population is also still comparatively homogenous, despite increasing immigration in the past three decades. In the end of year 2021, 12% of the whole population in the region of Southwest Finland was of foreign background, whereas the corresponding figure for the whole country was 8.5% (Statistics Finland: https://pxdata.stat.fi/PxWeb/pxweb/en/StatFin/StatFin__vaerak/statfin_vaerak_pxt_11rt.px/). The Finnish population is genetically somewhat distinct from most European populations. Yet the results of the genetic studies presented in this study were not attributable to the distinctive Finnish Disease Heritage that is mostly associated with very rare disorders. Examples of relatively common genetic disorders for which the population prevalence in Finland is distinct from that generally observed in Europe include the hexanucleotide repeat expansion in *C9orf72* (higher prevalence in Finland) and Friedreich ataxia (lower prevalence in Finland), which were not included in this study.

We had access to original individual medical records; data of each of the identified 844 patients were collected and confirmed individually. Finland and the other Nordic countries in Europe share a relatively generous public healthcare system, in which individual economic constraints and private insurance status do not affect access to advanced medical diagnostics and care. This is why our results probably represent the diagnostic needs of the population reasonably well compared to healthcare systems where access to costly advanced testing is more variable or limited [32]. There are, however, few previous reports of the utility and yield of NGS panels and exome sequencing studies for neurological disorders in Nordic countries [17, 31]. It is also worth pointing out that this study represents the actual diagnostic use of genetic testing at a Nordic European university hospital. In many other countries, the structure of the healthcare system and patient data storage systems do not enable this kind of study.

Moreover, in many countries even within Europe, the availability of advanced genetic tests such as NGS panels and exome studies remains very limited because of economic constraints.

In the present study, the yearly number of panels and exomes (CES and WES) was variable. The use of NGS panels was highest in 2016 ($N = 69$); during the years 2010–2021, the total number of genetic tests, including single gene analyses, somewhat decreased. This may result from the increased use of wider NGS panels and exome analyses and the related decrease in the use of multiple single gene analyses. There may also be random variation between years related to the fairly modest total number of genetic investigations. The timeline of this study was still relatively short, and based on our clinical experience, a major increase in the use of exome investigations at TUH and elsewhere in Finland has taken place only after the year 2021.

Various commercial gene panels provided by companies based in Finland, elsewhere in Europe and in the United States have been used at TUH. The details of the panel composition for a certain condition (e.g., a neuropathy panel and an ataxia panel) have varied based not only on the provider but also importantly on when the study was performed (i.e., more genes and pathogenic variants in more recent studies). During the period described in this study, gene panels were commonly used for nonsyndromic, more clearly defined phenotypes, such as ataxia, epilepsy or peripheral neuropathy, and could include dozens (e.g., 30–70) of genes. In recent times, in-house testing was increasingly used.

The overall diagnostic yield of NGS-based analysis (including panels and exomes) in our study was in line with the yield of exome studies in adult patients but somewhat lower than that reported in general (often mostly paediatric) studies [5, 17, 19, 32–34]. The challenge with gene panels, when ordered from different providers, is that the exact panel content may vary considerably. There is also a variety in how well different laboratories are updating their panels. At TUH, all gene panel studies are now in-house, and thus, data are more comparable. We estimate that in recent times, the proportion of in-house testing amounts to 95%. All gene panels and exome studies are now performed in-house. Only certain specific tests such as RNA studies, some repeat expansion tests or WGS studies are performed by an outside provider.

One potential explanation for the observed differences in diagnostic yields might be that more limited genetic tests, such as clinical exome analysis and panel testing, have been used more often to rule out genetic aetiology for the symptoms of the patient, and the more comprehensive WES was performed only when a genetic diagnosis was considered more likely. However, our data do not enable firm conclusions, as any pretest evaluation for the likelihood of detecting a genetic condition is typically not available for these studies. In some cases, partial information or misinterpretation of the clinical phenotype may have led to poor choices of genetic investigations or difficulties in the interpretation of results. It is also good to note that in this study, the yield consisted of groups of 'positive' diagnostic findings, 'negative' findings when no relevant variant was found and VUSs. Many previous studies of neurological genetic analyses have not reported the amount of VUSs, although they are increasingly reported recently [19]. In TUH, there are regular multidisciplinary team (MDT) meetings where the VUS findings of our in-house laboratory are discussed. MDT work has improved our VUS analysis considerably. At TUH, we obtain written consent for secondary findings in our exome analyses. Incidental findings are reported if considered beneficial for the patients, although laboratories outside TUH may have their own criteria for reporting incidental findings and secondary findings.

The classification of variants changes with time. With more genetic testing, more novel variants are reported. Any exome or panel analysis may result in VUSs that require further investigations, for example, segregation studies [1, 20].

The yield of exome has been reported at around 25%–30% when investigating Mendelian disorders [5, 18, 21, 35]. Incidental findings in WES have been reported at 5%–13% [5, 18, 21]. In children with a large variety of suspected Mendelian disorders, the yield of CES was reported at 23%. Amongst these, 3% had secondary findings reported by the recommendations of the ACMG [36]. If the genetic analysis using a panel or exome data does not result in a diagnosis, it is often worthwhile to reanalyse the data after a few years as knowledge of disease-causing genes and pathogenic variants increases. Studies suggest that such reanalysis may result in diagnostic yield increases by 10% [35]. As to the classification of detected variants, we acknowledge as a limitation of our study that the current ACMG classification was not in use for the earliest years in this study (before 2015).

Up to a third of patients with genetic neuromuscular disorders do not present with the phenotype typically associated with their gene variant. This is why the diagnostic yield is higher when a higher number of relevant genes are analysed [11, 21]. The diagnostic yields in our study (Table 2) were similar to those previously reported [9, 11, 21–23]. There were 91 genetic analyses of neuromuscular diseases. All investigated genetic testing techniques combined ($N = 75$) provided a positive yield of 39%. The highest yield came from WES (67%).

Epilepsy is a neurologic disorder with significant clinical and aetiological heterogeneity [1]. In our study, there were 60 genetic tests made for epilepsy. The overall yield of all investigated genetic testing techniques ($N = 32$) was surprisingly low (13%), compared to the previously reported 36% yield [22]. In the previous reports, the diagnostic yield has been reported as higher on selected subgroups such as childhood-onset epilepsy or epilepsy with comorbid ID/DD [4, 6, 10, 19]. One plausible explanation might be that our cohort consisted only of patients aged at least 16 years, and most of those patients with a severe phenotype (epilepsy and ID) were probably already diagnosed in paediatric care. The genetic cause of epilepsy is more likely with earlier disease onset [4]. However, in the present work, data on the age of onset for epilepsy were not collected. In our study, the molecular diagnostic yield for DD/ID (Table 2) did not differ from that in previous reports [10, 22, 37].

In our study, there were 84 genetic analyses made for ataxia. However, as genetic ataxia is commonly related to trinucleotide expansion disorders such as SCAs and ataxia related to *RFC1* and *FGF14* genes that require different methods for testing, the

NGS results here do not give a complete picture of genetic investigations performed in diagnosing ataxia [29, 38]. Since our focus was on the use and diagnostic rate of gene panels and exome studies, we do not present data on SCA, Friedreich ataxia and *RFC1* studies.

During the past decades, the landscape of available genetic methods in the investigation of genetic neurological disease has changed considerably. In clinical practice, the utilisation of various genetic investigations is variable and affected by several factors such as the availability of tests, the economic possibilities of utilising genetic investigations and the expertise needed to choose the appropriate tests according to the clinical questions and to interpret the results.

Constant development in the available methods of genetic diagnostic testing has considerably changed the diagnostic utility of various tests. In the future, optical genome mapping and long-read sequencing will bring forth new changes. Systematic studies and a good understanding of the genetic causes of neurological disease are needed to improve the utilisation and diagnostic yield of genetic tests used to investigate adult patients with neurological disease [39].

5 | Conclusion

The use of exome sequencing in the molecular diagnostics of neurological disease has increased rapidly in recent times. The shift from the use of more targeted genetic testing to the use of large-scale investigations advances slowly, but the trend is clear. More extensive testing is becoming the standard of care already in the first line of genetic investigations, particularly in the more affluent societies. During the next 10 years, further advances in the field of genetic diagnostics can be expected. In cases where variants in several hundreds of genes may underlie the same phenotype, an approach with early broad genetic testing will ultimately result in faster and cheaper diagnoses. Amongst neurological disorders, the NGS-based techniques including panels and exome studies had different diagnostic yields between patient groups. Our findings suggest that WES is most useful in patients with DD/ID and in those with neuromuscular disorders. Patients with severe epilepsy are often diagnosed already in paediatric care. Many patients with ataxia remain without a genetic diagnosis, as was the case in the present study. However, as a limitation regarding ataxia diagnostics, we acknowledge that repeat expansion disorders are common in ataxia, but these were not investigated in this study. In the future, new techniques such as long-read sequencing and analysis of intronic variants are likely proven useful. Overall, further work is needed to determine the optimal genetic testing strategy for many neurological patient groups.

Author Contributions

M.K.H. and M.H.M. shared last authorship.

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Ethics Statement

This register-based study was covered by the TUH research permission T04/010/22 and followed the principles of the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared.

References

1. I. Krey, K. Platzer, A. Esterhuizen, et al., "Current Practice in Diagnostic Genetic Testing of the Epilepsies," *Epileptic Disorders* 24, no. 5 (2022): 765–786, https://doi.org/10.1684/epd.2022.1448.

2. A. Pyle, T. Smertenko, D. Bargiela, et al., "Exome Sequencing in Undiagnosed Inherited and Sporadic Ataxias," *Brain* 138 (2015): Pt 2276–283, https://doi.org/10.1093/brain/awu348.

3. N. Schuermans, H. Verdin, J. Ghijesels, et al., "Exome Sequencing and Multigene Panel Testing in 1,411 Patients With Adult-Onset Neurologic Disorders," *Neurology: Genetics* 9, no. 3 (2023): , https://doi.org/10.1212/NXG.0000000000200071e200071.

4. D. McKnight, S. L. Bristow, R. M. Truty, et al., "Multigene Panel Testing in a Large Cohort of Adults With Epilepsy: Diagnostic Yield and Clinically Actionable Genetic Findings," *Neurology: Genetics* 8, no. 1 (2022): e650, https://doi.org/10.1212/NXG.0000000000000650.

5. Y. Yang, D. M. Muzny, J. G. Reid, et al., "Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders," *New England Journal of Medicine* 369, no. 16 (2013): 1502–1511, https://doi.org/10.1056/NEJMoa1306555.

6. B. R. Sheidley, J. Malinowski, A. L. Bergner, et al., "Genetic Testing for the Epilepsies: A Systematic Review," *Epilepsia* 63, no. 2 (2022): 375–387, https://doi.org/10.1111/epi.17141.

7. S. Srivastava, J. A. Love-Nichols, K. A. Dies, et al., "Meta-Analysis and Multidisciplinary Consensus Statement: Exome Sequencing Is a First-Tier Clinical Diagnostic Test for Individuals With Neurodevelopmental Disorders," *Genetics in Medicine* 21, no. 11 (2019): 2413–2421, https://doi.org/10.1038/s41436-019-0554-6.

8. S. J. Beecroft, P. J. Lamont, S. Edwards, et al., "The Impact of Next-Generation Sequencing on the Diagnosis, Treatment, and Prevention of Hereditary Neuromuscular Disorders," *Molecular Diagnosis & Therapy* 24, no. 6 (2020): 641–652, https://doi.org/10.1007/s40291-020-00495-2.

9. L. Gonzalez-Quereda, M. J. Rodriguez, J. Diaz-Manera, et al., "Targeted Next-Generation Sequencing in a Large Cohort of Genetically Undiagnosed Patients With Neuromuscular Disorders in Spain," *Genes (Basel)* 11, no. 5 (2020): 539, https://doi.org/10.3390/genes11050539.

10. K. L. Helbig, K. D. Farwell Hagman, D. N. Shinde, et al., "Diagnostic Exome Sequencing Provides a Molecular Diagnosis for a Significant Proportion of Patients With Epilepsy," *Genetics in Medicine* 18, no. 9 (2016): 898–905, https://doi.org/10.1038/gim.2015.186.

11. V. Nigro, and M. Savarese, "Next-Generation Sequencing Approaches for the Diagnosis of Skeletal Muscle Disorders," *Current Opinion in Neurology* 29 (2016): 621–627, https://doi.org/10.1097/WCO.0000000000000371.

12. K. W. P. Ng, H.-L. Chin, A. X. Y. Chin, and L.-M. D. Goh, "Using Gene Panels in the Diagnosis of Neuromuscular Disorders: A Mini-Review," *Frontiers in Neurology* 13 (2022): 997551, https://doi.org/10.3389/fneur.2022.997551.

13. K.-R. Dias, R. Shrestha, D. Schofield, et al., "Narrowing the Diagnostic Gap: Genomes, Episignatures, Long-Read Sequencing, and Health Economic Analyses in an Exome-Negative Intellectual Disability Cohort," *Genetics in Medicine* 26, no. 5 (2024): 101076, https://doi.org/10.1016/j.gim.2024.101076.

14. J. Rexach, H. Lee, J. A. Martinez-Agosto, A. H. Németh, and B. L. Fogel, "Clinical Application of Next-Generation Sequencing to the Practice of Neurology," *Lancet Neurology* 18, no. 5 (2019): 492–503, https://doi.org/10.1016/S1474-4422(19)30033-X.

15. C. J. Record, and M. M. Reilly, "Lessons and Pitfalls of Whole Genome Sequencing," *Practical Neurology* 24, no. 4 (2024): 263–274PMID: 38548322, https://doi.org/10.1136/pn-2023-004083.

16. D. T. Miller, K. Lee, N. S. Abul-Husn, et al., "ACMG SF v3.2 List for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing: A Policy Statement of the American College of Medical Genetics and Genomics (ACMG)," *Genetics in Medicine* 25, no. 8 (2023): , https://doi.org/10.1016/j.gim.2023.100866.

17. M. T. Sainio, J. Aaltio, V. Hyttinen, et al., "Effectiveness of Clinical Exome Sequencing in Adult Patients With Difficult-to-Diagnose Neurological Disorders," *Acta Neurologica Scandinavica* 145, no. 1 (2022): 63–72, https://doi.org/10.1111/ane.13522.

18. Y. Yang, D. M. Muzny, F. Xia, et al., "Molecular Findings Among Patients Referred for Clinical Whole-Exome Sequencing," *JAMA - Journal of the American Medical Association* 312, no. 18 (2014): 1870–1879, https://doi.org/10.1001/jama.2014.14601.

19. A. Stefanski, Y. Calle-López, C. Leu, E. Pérez-Palma, E. Pestana-Knight, and D. Lal, "Clinical Sequencing Yield in Epilepsy, Autism Spectrum Disorder, and Intellectual Disability: A Systematic Review and Meta-Analysis," *Epilepsia* 62, no. 1 (2021): 143–151, https://doi.org/10.1111/epi.16755.

20. C. Marques Matos, I. Alonso, and M. Leão, "Diagnostic Yield of Next-Generation Sequencing Applied to Neurological Disorders," *Journal of Clinical Neuroscience* 67 (2019): 14–18, https://doi.org/10.1016/j.jocn.2019.06.041.

21. K. Retterer, J. Juusola, T. M. Cho, et al., "Clinical Application of Whole-Exome Sequencing Across Clinical Indications," *Genetics in Medicine* 18, no. 7 (2016): 696–704, https://doi.org/10.1038/gim.2015.148.

22. H. Lee, J. L. Deignan, N. Dorrani, et al., "Clinical Exome Sequencing for Genetic Identification of Rare Mendelian Disorders," *JAMA - Journal of the American Medical Association* 312, no. 18 (2014): 1880–1887, https://doi.org/10.1001/jama.2014.14604.

23. S. Mellone, C. Puricelli, D. Vurchio, et al., "The Usefulness of a Targeted Next Generation Sequencing Gene Panel in Providing Molecular Diagnosis to Patients With a Broad Spectrum of Neurodevelopmental Disorders," *Frontiers in Genetics* 13 (2022): 1–11, https://doi.org/10.3389/fgene.2022.875182.

24. A. Mainali, T. Athey, S. Bahl, et al., "Diagnostic Yield of Clinical Exome Sequencing in Adulthood in Medical Genetics Clinics," *American Journal of Medical Genetics. Part a* 191, no. 2 (2023): 510–517, https://doi.org/10.1002/ajmg.a.63053.

25. A. H. Németh, A. C. Kwasniewska, S. Lise, et al., "Next Generation Sequencing for Molecular Diagnosis of Neurological Disorders Using Ataxias as a Model," *Brain* 136 (2013): pt 103106–3118, https://doi.org/10.1093/brain/awt236.

26. M. Sun, A. K. Johnson, V. Nelakudti, et al., "Targeted Exome Analysis Identifies the Genetic Basis of Disease in Over 50% of Patients With a Wide Range of Ataxia-Related Phenotypes," *Genetics in Medicine* 21, no. 1 (2019): 195–206, https://doi.org/10.1038/s41436-018-0007-7.

27. F. M. Snoeijen-Schouwenaars, J. S. van Ool, J. S. Verhoeven, et al., "Diagnostic Exome Sequencing in 100 Consecutive Patients With Both Epilepsy and Intellectual Disability," *Epilepsia* 60, no. 1 (2019): 155–164, https://doi.org/10.1111/epi.14618.

28. V. Juvonen, S. M. Kulmala, J. Ignatius, M. Penttinen, and M. L. Savontaus, "Dissecting the Epidemiology of a Trinucleotide Repeat Disease - Example of FRDA in Finland," *Human Genetics* 110, no. 1 (2002): 36–40Epub 2001 Nov 14, https://doi.org/10.1007/s00439-001-0642-x.

29. A. Cortese, R. Simone, R. Sullivan, et al., "Biallelic Expansion of an Intronic Repeat in _RFC1_ Is a Common Cause of Late-Onset Ataxia," *Nature Genetics* 51, no. 4 (2019): 649–658, https://doi.org/10.1038/s41588-019-0372-4.

30. D. Pellerin, M. C. Danzi, C. Wilke, et al., "Deep Intronic FGF14 GAA Repeat Expansion in Late-Onset Cerebellar Ataxia," *New England Journal of Medicine* 388, no. 2 (2023): 128–141, https://doi.org/10.1056/NEJMoa2207406.

31. Q. L. Holla, Ø. L. Busk, K. Tveten, et al., "Clinical Exome Sequencing-Norwegian Findings," *Tidsskrift for den Norske laegeforening: tidsskrift for praktisk medicin, ny raekke* 135, no. 20 (2015): 1833–1837, https://doi.org/10.4045/tidsskr.14.1442.

32. T. M. Bardakjian, I. Helbig, C. Quinn, et al., "Genetic Test Utilization and Diagnostic Yield in Adult Patients With Neurological Disorders," *Neurogenetics* 19, no. 2 (2018): 105–110, https://doi.org/10.1007/s10048-018-0544-x.

33. J. E. Posey, J. A. Rosenfeld, R. A. James, et al., "Molecular Diagnostic Experience of Whole-Exome Sequencing in Adult Patients," *Genetics in Medicine* 18, no. 7 (2016): 678–685, https://doi.org/10.1038/gim.2015.142.

34. M. Walsh, K. West, J. A. Taylor, et al., "Real World Outcomes and Implementation Pathways of Exome Sequencing in an Adult Genetic Department," *Genetics in Medicine* 24, no. 7 (2022): 1536–1544, https://doi.org/10.1016/j.gim.2022.03.010.

35. A. M. Wenger, H. Guturu, J. A. Bernstein, and G. Bejerano, "Systematic Reanalysis of Clinical Exome Data Yields Additional Diagnoses: Implications for Providers," *Genetics in Medicine* 19, no. 2 (2017): 209–214, https://doi.org/10.1038/gim.2016.88.

36. J. R. Murrell, A. M. I. Nesbitt, S. W. Baker, et al., "Molecular Diagnostic Outcomes From 700 Cases: What Can We Learn From a Retrospective Analysis of Clinical Exome Sequencing?," *Journal of Molecular Diagnostics* 24, no. 3 (2022): 274–286, https://doi.org/10.1016/j.jmoldx.2021.12.002.

37. J. de Ligt, M. H. Willemsen, B. W. M. van Bon, et al., "Diagnostic Exome Sequencing in Persons With Severe Intellectual Disability," *New England Journal of Medicine* 367, no. 20 (2012): 1921–1929, https://doi.org/10.1056/NEJMoa1206524.

38. A. Mundwiler, and W. G. Shakkottai, "Autosomal-Dominant Cerebellar Ataxias," *Handbook of Clinical Neurology* 147 (2018): 173–185, https://doi.org/10.1016/B978-0-444-63233-3.00012-9.

39. M. W. Waung, F. Ma, A. G. Wheeler, C. C. Zai, and J. So, "The Diagnostic Landscape of Adult Neurogenetic Disorders," *Biology* 12, no. 12 (2023): 1459, https://doi.org/10.3390/biology12121459.

Supporting Information

Supporting Information 1. List of variants from NGS tests.

Supporting Information 2. Patients with one or more than one NGS-based test or single gene test.