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The evolution of comprehensive genetic analysis in neurology: Implications for precision medicine

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SUMMARY

Technological advancements have facilitated the availability of reliable and thorough genetic analysis in many medical fields, including neurology. In this review, we discuss currently applied technologies for monogenic neurological disorders analysis focusing on the value of the selection of the appropriate genetic test to assist an accurate disease diagnosis. Moreover, the applicability of comprehensive analysis via NGS for various genetically heterogeneous neurological disorders is reviewed, revealing its efficiency in clarifying a frequently cloudy diagnostic picture and delivering a conclusive and solid diagnosis that is essential for the proper management of the patient. The feasibility and effectiveness of medical genetics in neurology require interdisciplinary cooperation among several medical specialties and geneticists, to select and perform the most relevant test according to each patient's medical history, using the most appropriate technological tools. In addition, the prerequisites for a comprehensive genetic analysis are discussed, highlighting the utility of using multigene NGS panels and obtaining a family history to increase the percentage of patients with informative genetic testing. Finally, we review the current applications of genetic analysis in the diagnosis and personalized treatment of neurological patients and the advances in the research and scientific knowledge of hereditary neurological disorders that are evolving the utility of genetic analysis towards the individualization of the treatment strategy.

Keywords: Next generation sequencing; Personalized treatment; Genetic analysis; Chromosomal microarrays; Neurogenetics

Abbreviations

NGS: Next Generation Sequencing

CMA: Chromosomal microarrays

VUS: Variant of Uncertain significance

CNV: Copy number Variation

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BACKGROUND

Neurological disorders comprise a group of heterogeneous entities characterized by the inappropriate function of central and peripheral nervous systems. They may present a variety of symptoms depending on the parts involved in the pathologic processes. The specific causes of neurological problems are variable and can include infections, injuries, lifestyle, or environmental factors. In recent years the importance of the genetic contribution to several neurological conditions has emerged. In line with the wide spectrum of signs and symptoms of such disorders, significant heterogeneity in the genetic etiologies responsible for the disease predisposition is also observed.

Moreover, recently, clinical and research interest focused on the prediction of diseases or phenotypes using the entire genome variation through Genome-wide association studies (GWAS) loci. Such an approach has added to the identification of new genes and genetic loci that contribute to an increased risk of several neurological diseases [1–3]. Nowadays, several neurological diseases such as Parkinson's, Alzheimer, migraines, and epilepsies are considered heterogeneous disorders with both monogenic and polygenic forms [4–7]. The polygenic forms are determined by the interaction of several independent genomic variants, which most likely also interact with non-genetic factors, such as environmental exposure and lifestyle choices [7,8].

Thus, through the compilation of GWAS studies, polygenic risk scores (PRS) have been constructed. These scores calculate the cumulative effect of low to intermediate risk variants in a patient population and estimate an individual's genetic liability to a trait or disease, calculated according to their genotype profile and relevant GWAS data. PRS is expected to be a prediction and risk stratification tool for identifying individuals with a higher predisposition to complex neurological diseases and holds promise to provide insights into the biological basis and the prediction of age-dependent clinical outcomes [8–10]

Several neurologic syndromes though are caused by highly penetrant but rare mutations with Mendelian pattern of inheritance, rendering molecular diagnosis mandatory. Guidelines recommend genetic evaluation for the identification of hereditary mutations, in case a genetic predisposition is suspected, for several neurological disorders such as Huntington, Parkinson's, Alzheimer, dystonia, spastic paraplegias, ataxias, and others. [11–16]. Nevertheless, given the increasing amount of information regarding the genetic etiology of neurological disorders, a revised version of the present guidelines that incorporate new molecular approaches should be considered where necessary.

The pattern of inheritance and the genetic loci implicated differ significantly among the disorders. Hence, the testing strategy used for genetic diagnosis should be tailored to fit the disorder and the disease phenotype. In clinical practice, it is imperative to make an appropriate genetic test selection to avoid unnecessary and inappropriate analyses that would result in diagnosis delays or even misdiagnosis in case of wrong test selection. A variety of analysis strategies is currently available and indicated for various neurological conditions with suspected monogenic genetic etiology, including, Chromosomal microarrays (CMAs), single-gene analysis strategies and multi-gene analysis using the Next Generation Sequencing technology (NGS).

Technologies used for the analysis of monogenic neurological disorders

Chromosomal microarrays (CMAs): Microarray-based genomic copy-number analysis is currently used for the detection of major structural anomalies. In cases of unexplained developmental disorder, mental retardation, autism spectrum disorder, or multiple congenital diseases, CMAs offer a much higher diagnostic efficiency (15% –20%) compared to the traditional G-band karyotype approach [17–19]. This is due to its higher sensitivity in submicroscopic chromosomal defects and duplications detection. The available data strongly support the application of CMA as the first cytogenetic diagnostic test instead of traditionally used techniques such as karyotyping and FISH for these patients [20]. Karyotype analysis should be limited to patients with apparent chromosomal syndromes, a family history of chromosomal rearrangement, or a history of multiple miscarriages [21,22]. The American Academy of Pediatrics also recommends CMA analysis in children with autism spectrum disorders, while, in case of a negative CMA result, subsequent fragile X analysis and RETT syndrome testing for females, are suggested [16]. Moreover, CMA analysis should be also considered for certain types of epilepsy, especially those with focal epilepsies or epileptic encephalopathies [23–25]. Importantly, if still a genetic diagnosis is not achieved more comprehensive analysis strategies such as Next Generation sequencing (NGS) are recommended.

Single gene analysis strategies: Genetic analysis of a single gene or even a single mutation should be applied for neurogenetic diseases whenever there is a clear association of a patient's phenotype with a specific genotype **Tab.1.** For example, Spinal muscular atrophy (SMA) is due to damaging mutations of the SMN1 gene (survival motor neuron 1) on chromosome 5q13. Analysis of this gene is mandatory and sufficient for the disease diagnosis [26]. Similarly, Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused by duplications (65–70% of patients) or point mutations in the dystrophin gene (dystrophinopathies) [27] Likewise, approximately 80% of patients with Hereditary neuropathy with liability to pressure palsy (HNPP) carry a deletion involving the peripheral myelin protein 22 gene (PMP22) and the same gene is duplicated in Charcot-Marie-Tooth (CMT) 1A patients. In a small proportion of patients, CMT1A is caused by *PMP22* point mutations [28-32].

Furthermore, more than 40 neurological disorders are caused by an increase in the number of repetitive short tandem DNA sequences. Hence it is estimated that 1 in 3.000 individuals carry disease-causing expansion repeats. Repeat expansions are usually formed during DNA replication, through slippage, due to mispairing between strands [33]. The number of repeats with a pathogenic effect varies between different disorders. As the number of repeats increases, the developing expansion changes the expression of the gene and/or the function of its product. In general, the larger the expansion the faster the onset of the

Tab. 1. Examples of monogenic disorders with single gene/locus genetic analysis recommended as a first-tier test (AD: Autosomal dominant, AR: Autosomal recessive, XLR: X-linked recessive, XLD: X-linked dominant).

Syndrome	Incidence	Inheritance	Gene	Prevalent Mechanism of disease	Proposed analysis method	Analysis in case of no diagnosis
SMA (29)	1/10.000	AR	SMN1/ SMN2	95% SMN1 exon deletions 5% mutations	SMN1/ SMN2 gene MLPA	SMN1 gene analysis
DMD/ BMD(30)	1-9 / 100.000	XL R	DMD	65-70% exon deletions	MLPA for DMD exon deletions	NGS gene analysis
HNPP (28,31)	1-9 / 100.000	AD	PMP22	PMP22 deletion	MLPA	NGS analysis for Neuropathies
CMT 1A (28)	1-5 / 10.000	AD	PMP22	>99% PMP22 duplication	MLPA	NGS for CMT-associated genes
Rett syndrome (RTT) (32)	1-9 / 100.000	XLD	MECP2	MECP2 mutations	MECP2 sequencing	DKL5, FOXG1 gene analysis by NGS

disease, and the more severe the disease becomes. Analysis of this type of alteration is carried out using targeted PCR-based molecular analysis of an individual locus, guided by the suspected clinical diagnosis **Tab.2.**

There are several examples of repeat expansion disorders where molecular analysis is recommended. Myotonic dystrophy type 1 (DM1) is caused by the increase in the number of triplet repeat (CTG) in the DMPK myotonic kinase gene. Myotonic dystrophy type 2 (DM2) type of the disease, which is much rarer, is caused by CCTG expansion in the nucleic acid-binding protein (CNBP) gene (previously known as zinc finger 9, ZNF9), and Fragile X syndrome is due to the repetition of CGG triplet in 5′UTR of the fragile X mental retardation 1(FMR-1) gene. In Friedreich's Ataxia, 98% of cases are due to GAA trinucleotide extension (> 66 repeats) in the first intron of the *FXN* gene in both alleles. The Huntington's chorea is due to an extension of the CAG triplet repetitions in exon 1 of the IT15 gene. Furthermore, up to 50% of Familial Amyotrophic lateral sclerosis (ALS) and 29% of FrontoTemporal dementia (FTLD) are due to an extension of a GGGGCC (G4C2) repeat at the 5 'UTR of the gene C9orf72 [34–36]. Autosomal dominant cerebellar disorders (ADCA) (SCA1, SCA2, SCA3, SCA6, SCA7, et al.) fall also under the spectrum of trinucleotide repeat disorders [37-48].

Next generation sequencing: Several neurological disorders have a wide spectrum of symptoms with variable severity, which makes their clinical identification difficult. Additionally, distinct genetic alterations in a gene can produce different effects on the encoded sequence, while the genetic background of each individual as well as environmental and other factors can modify the effects of a genetic variation. Moreover, many neurological disorders are not fully described, and new disorders are being constantly described. From a genetic point of view, these disorders are characterized by genetic heterogeneity of phenotype-genotype correlation. This fact is reflected in the frequent association of many different genes and genetic loci with a certain genetic disorder [49]. Furthermore, different genetic alterations occurring in a particular gene can be associated with distinct neurological disorders. ATP1A3-Related Disorders is an example of such a pleiotropic phenomenon since different alterations in the ATP1A3 gene have been detected in Rapid-onset dystonia-parkinsonism, as well as alternating hemiplegia in children and CAPOS syndrome [50].

In addition, the incomplete penetrance of various genetic alterations, the phenotypic overlapping between neurologic disorders, and differences in the severity of the symptoms present in each case increase the complexity of genomic diagnosis [51,52]. A significant number of genes should be evaluated whenever a neurological disease is suspected, as indicated from the number of genes related to neurological conditions, in available databases of human genes and genetic diseases' phenotypes such as the Online Mendelian Inheritance in Man (OMIM) and the Human Phenotype Ontology (HPO) [53,54]

Advances in molecular technologies enabled the implementation of advanced genomic techniques in a variety of rare genetic diseases including neurological disorders [55]. The increasing use of Next Generation Sequencing technology permits the analysis of multiple genes simultaneously at a low cost. In addition, advances in computational and bioinformatics sciences enabled data management and interpretation of the results obtained. Therefore, the availability of such wide genomic analysis platforms by a growing number of laboratories provided

Tab. 2. Examples of repeat expansion diseases.

Syndrome	Gene	Inheritance	Repeat	Incidence	Normal	Disease
Fragile X Syndrome (38)	FMR1	XLD	CGG	1-5/10.000	6-55	>230
Friedreich Ataxia (39)	FXN	AR	GAA	1-9/100.000	7-34	>66
Myotonic dystrophy type 1(40)	*DMPK*	AD	CTG	1-5/10.000	5-34	>50
Myotonic dystrophy type 2(41)	CNBP	AD	CCTG	1-9 / 100 000	<30	75-11000
Huntington Disease (42)	IT15	AD	CAG	1/100.000	6-36	>36-121
ALS/FTLD (43)	C9orf92	AD	GGGGCC	1-9/100.000	<24	>60
Spinocerebellar Ataxia Type 1(44,45)	ATXN1	AD	CAG	1/100.000	6-38	39-80
Spinocerebellar Ataxia Type 2(44,45)	ATXN2	AD	CAG	1-2/100,000	16-30	36-52
Spinocerebellar Ataxia Type 3(44,45)	ATXN3	AD	CAG	1-9 / 100.000	14-40	60-85
Spinocerebellar Ataxia Type 6	CACNL1A4	AD	CAG	1-9 / 1.000.000	5-20	21-28
Spinocerebellar Ataxia Type 7(44,45)	*ATXN7*	AD	CAG	1-9 / 1.000.000	7-19	37-220
Spinocerebellar ataxia type 17(44,46)	*TBP*	AD	CAG	<1 / 1.000.000	25–41	>48
X-linked spinal and bulbar muscular atrophy (47)	AR	XLR	CAG	Ultra-Rare	10-36	>40
Dentatorubral-pallidoluysian atrophy (48)	*ATN1*	AD	CAG	1-9 / 1.000.000	7-35	>48

Tab. 3. Number of HPO and OMIM-driven genes implicated in various neurological diseases.

Disease	No of genes in OMIM	No of genes in HPO	HPO Link
Parkinsonism	155	119	HP:0001300
Amyotrophic Lateral Sclerosis (ALS)	200	43	HP:0007354
Dementia	200	184	HP:0000726
Migraine	94	113	HP:0002076
Peripheral Neuropathy	200	658	HP:0009830
Seizure	200	2736	HP:0001250
Spastic Paraplegia	200	193	HP:0001258
Muscular dystrophy	200	141	HP:0003560
Autism Spectrum Disorder	200	555	HP:0000729
Leukoencephalopathy	136	189	HP:0002352
Leukodystrophy	129	83	HP:0002415
Myopathy	200	326	HP:0003198

the opportunity to increase our understanding of these disorders while new genes and genetic alterations are constantly being associated with an increased risk of neurological disorders.

In general, several neuromuscular disorders, neuropathies, and myopathies need multigene evaluation. It is also required for the genetic study of hereditary spastic paraplegias (HSP) and disorders related with hereditary forms of dementia, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (PD). Moreover, NGS analysis is also required for several neurodevelopmental disorders and epilepsies **Tab. 4.** Multigene analysis is also mandatory whenever a previous single gene analysis returns negative results. This is often in the cases of ALS and FTD with a negative result for the C9 or f72 expansion, or in patients with a PMP22 suspected disorder without an alteration identified.

Several NGS strategies have been applied in neurology. Analysis of 50-100 genes related to a specific disorder was initially preferred, mainly due to the targeted and thus easier analysis required and to their lower cost. However, improvements in the NGS platforms' technology and the increase in their sequencing capacity permitted the more accurate and faster analysis of a larger number of gene simultaneously. Currently, NGS approaches are focused on the sequencing of the coding regions and adjacent intronic regions of either the 5000-7000 clinically relevant genes (Clinically Exome Sequencing), or even of the about 20.000 genes that are known to be protein-producing (Whole Exome Sequencing, WES) [55]. Following the sequencing process, analysis can be more extensive, or it can only include the genes related to a patient's phenotype, through appropriate gene selection and the creation of virtual NGS panels related to the phenotype attributed [55-68]. This approach enables the analysis of more genes related to a specific phenotype, while the genetic information obtained can always be accessed in case a new gene is related to the patient's disease or in case of the manifestation of a new disease in the individual tested. Furthermore, the comprehensive nature of the analysis provides the opportunity to apply a more expanded evaluation, in case of initially negative results, considering the possibility of other genetic disorders with similar phenotypic manifestations, increasing the diagnostic yield.

Obtaining high accuracy and sensitivity of NGS genomic analysis is of great importance. Hence, validated NGS methodologies should be used, capable of detecting all types of genetic variations, videlicet, Single Nucleotide Variations, small insertions, and deletions as well as intragenic Copy Number Variations (CNVs). For a long time, the platform of choice to detect genome-wide CNVs has traditionally been CMA, while multiplex ligation-dependent probe amplification (MLPA) was applied for the detection of smaller intergenic CNVs. However, due to bioinformatics and methodological improvements, those platforms tend to be replaced by highly sensitive NGS technologies. This is very important for the genetic diagnosis of neurological disorders, since intragenic CNVs represent a large percentage of the genomic variations observed. A recent NGS study for example indicated that in neuropathies, Charcot Marie Tooth, neuromuscular disorders, and epilepsies, CNVs account for 13-46% of the pathogenic variants detected [69].

Nowadays there is also growing evidence that newer sequencing technologies could contribute to the increase of the diagnostic rate of NGS in undiagnosed cases. Whole Genome Sequencing (WGS) technology is not limited to the analysis of coding regions of the genome but also gives insight into deep intronic and intergenic regions that could contribute to disease development. In addition, the PCR-free nature of the methodology permits a more accurate CNV analysis and can also detect short tandem DNA sequences repeat expansions which account for a substantial percentage of neurological conditions as well as variations in mitochondrial DNA, that usually remain undiagnosed [70,71].

Variant interpretation

An important component of the NGS analysis is its ability to perform appropriate variant classification and interpretation. Analysis can become demanding because when a big number of genes are analyzed, the number of findings that are detected and require classification of their pathogenicity increases exponentially. For example, it is expected that when a WES analysis is performed, it will return a median of about 50.000 variations compared to a reference genome, and of those almost 1700 have a minor allele frequency in the general population of less

Tab. 4. Diagnostic yield of NGS analysis in several neurological diseases.

Disease	Method	No of patients	Study	% of patients with VUS	% of patients with P/LP variant
Central Nervous System					
Alzheimer-early onset	50 gene NGS panel	67	Giau et al. (56)	Not Reported	6%
Neurodevelopmental disorders		1672	Yang Y et al. 2014(57)	Not Reported	25%
Early-onset and familial parkinsonism	40 gene NGS panel, repeat-primed PCR, and WES	571	Lin C et al. 2019 (58)	Not Reported	13.5%
Ataxia	285 gene panel	377	Galatolo et al. 2021(59)	15.6%	33.2%
Movement disorders	127 gene panel	378	Montaut et al. 2018 (60)	15.9%	22%
Epilepsy	89-189 genes	2008	McKnight et al 2022(61)	10.09%	19%
Dementia/leukodystrophy	WGS	32	Cochran et al. 2019(62)	Not Reported	50%
Dementia/leukodystrophy	WES	71	Vanderver et al. 2016(63)	4.2%	38%
Dementia/leukodystrophy	WGS	41	Helman et al. 2020 (64)	4.9%	29.3%
Dystonia	WGS	111	Kumar et al. 2019 (65)	28.8%	11.7%
Dystonia	18 gene Targeted panel	1910	Winder et al. 2020 (66)	11.78%	7.9%
Spastic Paraplegia	45 gene panel	2129	Winder et al. 2020 (66)	36.97%	13.9%
Peripheral Nervous System					
ALS	44 gene panel	100	Shepheard et al.(67)	21%	21%
Neuropathy	72 gene panel	11302	Winder et al. 2020 (66)	45.72%	12%
Myopathy	52 gene panel	1082	Winder et al. 2020 (66)	52.03%	9.61%
Congenital Myasthenia	16 gene panel	650	Winder et al. 2020 (66)	22.46%	4.31%

than 1% [72]. However, only a minority of such variants are causative of monogenic disease; most are part of normal human variation or may contribute to an increased or decreased risk of multi-factorial disease. Thus, the decision about their clinical significance is demanding and requires advanced bioinformatics tools for their appropriate categorization [73].

In 2015 the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published standards and guidelines for the interpretation of sequence variants [74]. They provided criteria and levels of evidence for the classification of the variants as "pathogenic", "likely pathogenic", "uncertain significance", "likely benign" or "benign".

Thus, the decision about the pathogenicity of each variant should be evaluated based on publicly available data from population and disease databases as well as published functional information. Computational data on the effect of the variant in protein function and other available data related to phenotype and segregation analysis results should also be considered in the annotation process. Subsequently, the variant should be classified in 5 classes of pathogenicity based on the available guidelines. It is also recommended that all assertions are classified with respect to a disease and inheritance pattern. This process should be dynamic permitting revaluation of the variant's pathogenicity in case new information becomes available in the future.

A big challenge of NGS analysis where multiple genes or exomes are sequenced simultaneously is that it produces a significant number of variants without a conclusive classification as pathogenic or benign. These variants are characterized as variants of uncertain significance (VUS) and should not be used in clinical decision-making according to the guidelines. As seen in **Tab. 3.** the number of VUS detected varies between different studies and depends on the number of genes analyzed and the stringency of the criteria used for their classification. A VUS could be reclassified in the future if additional information, not available at the time of the original classification, becomes accessible. This can be achieved for example using segregation analysis to test out if it segregates with the disease in other family members of the proband, or in case of a splicing variant using RNA analysis to clarify its effect in the splicing process. Furthermore, the increasing use of NGS technology assists in the accumulation of additional information about genes and variants, and thus will facilitate VUS reclassification in the future.

To this end, of great utility in accelerating VUS

reclassification is the data sharing between laboratories performing such tests, which could enrich the available information concerning the effect of a variant. Thus, it is important to report all variants detected in publicly available databases such as clinvar which is a repository where medically important variants and phenotypes relation is reported [75]. All publicly available information about mutation carriers' phenotype, data about the segregation of the variant in the families, as well as any existing functional analysis data should be considered in the VUS classification process and reported. In addition, laboratories should be able to keep a register of variants detected and periodically re-inspect and reclassify them when new information becomes available [74].

ClinVar data analysis

The Clinical Variant database (ClinVar) integrates knowledge concerning genetic variation and its association with human disease. Examining these data may provide insight into the genes implicated in various diseases and the detected mutation type.

If we examine the ClinVar data, there are 1,502,769 variation records with submitted interpretations (Unique variation records with interpretations) specific to one gene (13,236 genes) until August 2022. Among these records, 857,794 variants concern 2208 genes associated with neurological diseases. Of the variants detected in Neurological disease associated genes, 341,511 were missense 117,908 were PVS1 which are defined as "null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss-of-function (LoF) is a known mechanism of disease" (PMID: 30192042). Of these 1,502,769 variation records, 136,708 have been classified as pathogenic (P), 55,202 as Likely Pathogenic (LP), 590,985 as VUS, 411,555 as Likely Benign (LB) and 227,282 as Benign (B), while for 65,505 variants there are Conflicting interpretations.

Of the genes with related variants in clinvar, 2208 are reported in HPO as associated with neurological diseases. In these genes, the classification rates were as follows: 82,040 P, 35,868 LP, 341,511 VUS, 244,150 LB, 107,036 B and 41,838 Conflicting.

In order to evaluate the rate and type of variants that have changed classification category, and thus strength of pathogenicity in clinvar, an analysis of the variants' reclassified by the same submitter was performed between 2016 (when ACMG criteria had already been published) and recently (August 2022), as previously described [76,77]

Between August 2016 and August 2022, 2,368,489 classifications using one of the five standard ACMG/AMP classification categories were submitted to ClinVar. By August 2022, only 2.94% (69,601/2,368,489) of these categories had been reclassified and updated in ClinVar by the submitter. Among these reclassifications, 18.4% (12,805/69,601) were moved to a higher classification category (VUS to LP/P, LP to P, LB/B to VUS, LB/B to LP/P), while 81.6% were downscaled. Of the five classification terms, 32,358 variants initially classified as VUS were reclassified (3.49%), with 16.73%. of the reclassification being upgraded to the P/LP category and 83.27% being downscaled as B/LB **Tab. 5.**

For genes related to Neurological disorders, between 2016 and 2022 1,277,845 classifications were submitted to ClinVar using one of the five standard ACMG/AMP classification terms. By August 2022, only 3.18% (40,632/1,277,845) of these classifications had been

Tab. 5. Summary of classification and reclassification from ClinVar (Aug 2016 – Aug 2022) (adapted from Harrison S, et al. [76].

Starting classification (n)	Percentage reclassified (n)	Reclassification type (n)	Percentage of initial classification group	Percentage of all reclassifications
Pathogenic (272,149)	0.62% (1,675)	P → LP (865)	51.6%	1.2%
		P → VUS (719)	42.9%	1.0%
		P → LB (36)	2.1%	0.05%
		P → B (55)	3.3%	0.08%
Likely pathogenic (109,220)	4.96% (5,420)	LP → P (3,991)	73.6%	5.7%
		LP → VUS (1,269)	23.4%	1.8%
		LP → LB (130)	2.4%	0.19%
		LP → B (30)	0.55%	0.04%
Uncertain significance (927,967)	3.49% (32,358)	VUS → P (2,382)	7.4%	3.4%
		VUS → LP (3,030)	9.4%	4.4%
		VUS → LB (20,066)	62.0%	28.8%
		VUS → B (6,880)	21.3%	9.9%
Likely benign (664,524)	4.50% (29,928)	LB → P (24)	0.08%	0.03%
		LB → LP (27)	0.09%	0.04%
		LB → VUS (3,272)	10.9%	4.7%
		LB → B (26,605)	88.9%	38.2%
Benign (394,629)	0.06% (220)	B → P (28)	12.7%	0.04%
		B → LP (8)	3.6%	0.01%
		B → VUS (43)	19.5%	0.06%
		B → LB (141)	64.1%	0.2%

(**Abbreviations:** B: Benign, LB: Likely benign, LP: Likely pathogenic, P: Pathogenic, VUS: Variant of uncertain significance)

reclassified by the submitter and updated in ClinVar. 19.58% (7,956/40,632) were moved to a higher classification category (VUS to LP/P, LP to P, LB/B to VUS, LB/B to LP/P), while 80.42% were downscaled. VUS were reclassified in 3.79% (19,077/503,258) of the cases, with 18.64% of the reclassified cases being assigned to a higher category P/LP and 81.36% being downscaled as B/LB **Tab. 6.**

Based on the clinvar data and as recent studies have demonstrated, the majority of VUS are finally downgraded to benign or likely benign [66,78]. Thus, clinicians should be very cautious with VUS management, because the erroneous use of such variants as pathogenic could have harmful consequences not just for the proband but also for his relatives who could receive false information concerning their probability of disease inheritance and whose clinical management could be erroneously altered through the cascade testing.

Genetic counseling

A genetic analysis referral should always be accompanied by appropriate genetic counseling for the patient and the family from an expert genetic counselor [79–81]. To this end, the pre-test genetic counseling is of major importance. The first step to appropriately evaluate the likelihood of a genetic cause of the disease should be the collection of all clinical information about the proband and the family. Thus, a pedigree is constructed with information for at least three generations, about pathological conditions in the family. A clear description of the aim of such an analysis should be provided to the person under examination and/or to his family and information about the genes analyzed should also be provided [82]. Moreover, in the case of a WES analysis, patients and/or the family should be informed about the possibility of choosing to analyze genes identified as clinically relevant by international guidelines and to which the findings are proposed to be reported, regardless of the reason for referral. Currently, the ACMG list for reporting secondary findings in clinical exome and genome sequencing includes 78 genes [81]. Furthermore, a clear view of the possible results of the analysis should be provided. It should be explained that the test could outcome in a positive, a negative, or a VUS result.

In case of a negative result, it should be explained, especially in case of a family history of the disease, that the genetic result cannot exclude the presence of a genetic cause for the disease. There are several reasons that could lead to the missing of a causative variant. For instance, this could be due to lack of evaluation of the gene involved s a result of missing knowledge about the genes implicated in the disease or due to the inability of the technology used to detect the causative variant for example deep intronic variants or certain types of CNVs). In this case, it should be explained that it is not possible to offer genetic predisposition analysis to family members to determine the risk of the disease. Therefore, all first-degree family members should continue to be considered at risk for the disease and undergo recommended family surveillance. Genetic testing may be reviewed in the future if new information is available on the possible genetic causes of the condition.

Whenever a VUS is detected, the management should be the same as with a negative result, properly informing the proband and family that there is still the possibility of an inherited neurological condition in the family. Therefore, proposed surveillance of the proband and the family members at risk should be followed.

When the genetic test reveals a positive result, then the gene mutation that causes the disease has been detected.

Tab. 6. Summary of classification and reclassification from ClinVar (Aug 2016 – Aug 2022) for genes associated with neurological disorders (adapted from Harrison S. et al. (76).

Starting classification (n)	Percentage reclassified (n)	Reclassification type (n)	Percentage of initial classification group	Percentage of all reclassifications
Pathogenic (135,762)	0.84% (1,143)	P → LP (599)	52.41%	1.47%
		P → VUS (471)	41.21%	1.16%
		P → LB (25)	2.19%	0.06%
		P → B (48)	4.20%	0.12%
Likely pathogenic (64,316)	5.24% (3,370)	LP → P (2,448)	72.64%	6.02%
		LP → VUS (831)	24.66%	2.05%
		LP → LB (75)	2.23%	0.18%
		LP → B (16)	0.47%	0.04%
Uncertain significance (503,258)	3.79% (19,077)	VUS → P (1,568)	8.22%	3.86%
		VUS → LP (1,987)	10.42%	4.89%
		VUS → LB (11,449)	60.01%	28.18%
		VUS → B (4,073)	21.35%	10.02%
Likely benign (381,351)	4.44% (16,922)	LB → P (11)	0.07%	0.03%
		LB → LP (13)	0.08%	0.03%
		LB → VUS (1,888)	11.16%	4.65%
		LB → B (15,010)	88.70%	36.94%
Benign (193,158)	0.06% (120)	B → P (11)	9.17%	0.03%
		B → LP (3)	2.50%	0.01%
		B → VUS (27)	22.50%	0.07%
		B → LB (79)	65.83%	0.19%

(**Abbreviations:** B: Benign, LB: Likely benign, LP: Likely pathogenic, P: Pathogenic, VUS: Variant of uncertain significance)

This finding verifies the genetic origin of the disease and has implications for its diagnosis. It aids in the care of patients, family cascade testing, and in some circumstances, the direction of therapy choices. Upon test completion, the genetic analysis findings should be thoroughly explained to those concerned and may lead, if necessary, to referral to other medical specialties for management recommendations, surveillance, and psychological support.

In some cases, the genetic analysis requested reveals negative results for a specific genetic disorder, but throughout the genetic counseling process, the geneticist may suggest the possibility of a different Mendelian disease. This became feasible due to the implementation of comprehensive NGS genomic tests, and the application of virtual panels, giving the possibility to analyze genes related to a disease suspected by the physician but also permitting the scanning of additional genes in case of a negative test, expanding the phenotypes covered by the analysis.

A paradigm of the importance of interdisciplinary collaboration, is the case of a 7-year boy referred to our laboratory with suspicion of Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) syndrome, based on the urine organic acid analysis. Sequencing analysis of the three genes (*ETFA, ETFB* and *ETFDH*) involved in such syndrome is sufficient for the diagnosis [83]. However, such analysis returned a negative result. However, based on the extensive family pedigree available, it was noticed that the boy was born from a consanguineous marriage between two first cousins, and he also presented other important phenotypic features, such as epilepsy, hypoglycemia, and hypothyroidism. The family history also indicated the possibility of an epileptic disorder with a recessive pattern of inheritance since a first cousin of the patient also presented epileptic seizures. Thus, a more expanded genomic analysis was proposed. Based on such analysis a homozygous pathogenic mutation in the CNTNAP2 gene was detected (c.1361_1362delAT, p.Asn454fs*24). The CNTNAP2 (Contactin Associated Protein 2) gene encodes a neuronal transmembrane protein of the neurexin family, important for the function of the vertebrate nervous system and associated with the autosomal recessive syndrome PTHSL1 (Pitt-Hopkins-like syndrome 1) [84]. This syndrome is a neurodevelopmental disorder characterized by mental retardation, speech problems, seizures, and behavioral disorders [85].

Furthermore, a pathogenic mutation was also detected in the G6PD, Glucose-6-phosphate dehydrogenase gene (c.1450C>T, p.Arg484Cys). This gene encodes an enzyme with an important role in cells' protection, especially red blood cells, from oxidative stress. Deficiency of the G6PD enzyme can lead to acute hemolytic anemia (AHA) after exposure to certain substances, such as aspirin, naphthalene, certain antibiotics and antimalarial drugs, and beans. G6PD deficiency follows the X-linked inheritance pattern [86]. Genetic diagnosis is important to avoid the AHA triggering factors. Therefore, the application of a more extensive genetic analysis than the one initially requested not only explained the proband's pathogenic features, especially those related to epilepsies but also provided information for a metabolic disease of which the family was not aware.

Genomic results reporting

A major part of the genetic counselling process concerns the reporting of the results of genomic diagnostic testing and thus international guidelines exist and should be followed [87]. The results should be reported in a clear comprehensible form for both patients and physicians. The report should include the reason for genetic testing referral and the genes analyzed based on the patient's phenotype. In case of an NGS genetic test, the rationale and databases used for gene selection should also be reported. The targeted regions by the assay should be clearly defined and if only coding regions and flanking intronic sequences are included in the analysis it should be clearly stated. The reference sequence used for the alignment and the relevant transcripts should be included. An accurate description of the NGS methodology applied should also be reported, including information about the platform used, the read depth and the assay's sensitivity and specificity in detecting various types of variation (including CNVs). Furthermore, information about the bioinformatic algorithms and the software used for variant calling and interpretation should be provided. All pathogenic or likely pathogenic mutations reported should be accurately described and the classification proposed should be fully justified with reference to the criteria used for the classification as recommended by the ACMG guidelines. Additional information provided should be the variant frequency in population databases and in mutation databases or bibliographic reports describing cases of affected individuals carrying the same alteration. A clear description of the gene(s) affected and the association with the patient phenotype should also be provided. In case of a VUS reported, the ACMG criteria used for its classification should also be reported as well as any *in silico* analysis available and the predicted effect in the protein's function. A clear statement that VUS should not be used for clinical decision making should also be included.

Clinical utility of genetic analysis

Genetic analysis is mandatory for several neurological diseases with suspicion of hereditary origin and could assist in better disease diagnosis, prognosis, and management.

Genetic analysis confirms the clinical diagnosis reliably and could reduce the need for more invasive procedures for diagnosis confirmation, such as muscle or nerve biopsies, which hold a modest but known possibility of morbidity. Furthermore, since many neurological disorders are genetically very heterogeneous, NGS analysis facilitates quick diagnosis by evaluating all possible disease-causing genes simultaneously. Obtaining a conclusive molecular diagnosis permits the clinician to set up appropriate, potentially life-saving surveillance or referrals. In certain circumstances, a confirmed molecular diagnosis may lead to modified medical management and treatment.

Thus, molecular diagnosis minimizes dilemmas regarding the management of differential diagnosis, especially in disorders where the symptoms are milder, such as in Myotonic Dystrophy Type 2 or the diagnosis is more demanding as in Spinal Muscular Atrophy (SMA) and Autosomal dominant Cerebellar Ataxia [88–90]. Another example where genetics can aid diagnosis is hereditary myopathies which are caused by mutations in various genes encoding proteins with significant roles in muscle structure and function. However, similar histopathological features may overlap in different hereditary myopathies with significant genetic heterogeneity and phenotypic pleiotropy, making difficult a specific diagnosis. In this regard, genetic analysis can facilitate better diagnosis and treatment [91].

Differential diagnosis is also important for the identification of various subtypes of the Charcot-Marie-Tooth (CMT) disease, as there is substantial overlap between the different forms. CMT should also be discerned from other diagnoses including inherited neuropathies, neuromuscular disorders such as distal myopathies and lower motor neuron disorders, and genetic disorders with CNS involvement such as spastic paraplegias, hereditary ataxias, and mitochondrial encephalopathies. The rate and clinical severity vary depending on the CMT subtype [92].

For many inherited disorders, the knowledge of the causative for a disorder mutation often provides the ability to predict its course. Hence it can be used as a prognostic biomarker of the disease progression. Further research is needed to determine how genetic analysis affects the prognosis of hereditary neurological disorders; however, it has been noted in several neurodegenerative diseases as well. In Parkinson's disease, for example, it has been recently shown that patients with LRRK2 mutations had longer survival rates compared to the wild-type ones. In contrast, those with an SNCA or GBA mutation had a shorter survival. [93]. In several repeat expansion disorders, the number of repeats is prognostic of the disease age of onset, and aggressiveness. An increased expansion repeat number is usually associated with earlier age of symptoms' initiation and shorter survival after the disease onset [37,40,94].

A positive genetic analysis result could be useful not only for the patients themselves but also for relatives at increased risk of developing the same disorder. Thus, cascade analysis of at-risk relatives for the variant detected in the proband should be offered and could lead to proper surveillance and management in case of a positive result. On the other hand, if the proband's pathogenic mutation is absent in the relative (s) tested, needless anxiety will be avoided.

Molecular diagnosis could also help reduce disease recurrence in families, especially in pediatric neurogenetic diseases, by using the option of prenatal or preimplantation genetic analysis, in order to prevent the inheritance of the pathogenic mutation within the family and thus avoid the disease occurrence in other family members [95].

In addition, the application of comprehensive NGS analysis to an increasing number of patients and medical conditions will provide a better insight into the genes involved in an increasing number of diseases with previously unknown genetic etiology. The information gained can then be applied to the new experimental approaches that are in development.

Utility of genetics in precision medicine in neurology

Precision medicine, also commonly referred to as personalized medicine, unlike the traditional one, places the patient in the center of health care, developing targeted diagnostic, therapeutic, and preventive strategies that consider differences in patients' genetic profiles as well as environmental factors. It uses our ever-evolving knowledge of how gene variability leads to differences in disease susceptibility and treatment response. A complex collection of data on patients' genetic profile, environment, and lifestyle, that could possibly affect response to a particular intervention is required, aiming to better target treatment and prevention. Precision medicine is not just about drugs. It is also about better understanding the biological mechanisms as well as the environmental factors that lead to disease development and affect all health care, from research to patient treatment and management [96,97].

The precision medicine approach has been enforced by the increased knowledge gained from the human genome obtained from the Human Genome Project, while advances and availability of new biomedical and informatics technologies enabled comprehensive genome analysis by NGS as well as data interpretation and storage. This had a major impact on the comprehension of the variability of patients' responses to various medical interventions and has led to new targeted drug development activities.

In the field of neurology already several gene alterations have been correlated to individualized treatment and diet interventions, enabling a more personalized approach based on each patient's genetic profile **Tab.4.** As new sequencing technologies are employed more often, it is anticipated that our understanding of the genetic pathways relating to disease prognosis and the prediction of response to intervention techniques will grow, further enabling the individualization of patients' medical care.

Targeted therapies in neurogenetic disease

In the era of targeted therapy, proper disease management necessitates the utilization of biomarkers that could inform prognosis, diagnosis, treatment monitoring, along with treatment selection. Particularly for the least, it is imperative to utilize appropriate predictive biomarkers. However, in neurogenetics, the term "predictive biomarker" is not well established, and in some instances, it is improperly used to designate biomarkers that foretell the onset or progression of the disease without regard to treatment.

The FDA-NIH Biomarker Working Group has

established the BEST (Biomarkers, EndpointS, and other Tools) Resource, aiming to clarify ambiguities regarding distinct biomarker type definition and utility in clinical practice and to highlight their role in medical product development. According to the BEST definition a predictive biomarker "is used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent"[98]. Hence, by definition, a predictive biomarker should be used to predict a disease's progression in correlation to a specific treatment selection.

Such types of biomarkers are currently applied successfully in the field of oncology and hematology where hereditary or somatic genetic alterations are targeted by specific treatment regimens and thus are used as predictive biomarkers for the identification of patients eligible to receive such targeted treatments [99–103]. Improved clinical benefits have been observed in gene-directed treatment strategies compared to unselected therapy interventions, for several malignancies [104–107]. Non-small cell lung cancer represents an example of the tumor type with the most biomarkers and targeted treatments available. Currently, the use of somatic gene mutation analysis is mandatory for determining the appropriate first or consequent lines of targeted treatment, while medical guidelines recommend genomically informed treatment decision-making [102–104]. Similarly, in ovarian breast and pancreatic cancers Breast Cancer genes 1 and 2 (BRCA1/2) mutations are used as predictive biomarkers to identify patients likely to respond to Poly (ADP-ribose) polymerase (PARP) inhibitors treatment [108].

Since the genomic analysis is increasingly applied to more patients, it is becoming evident that several gene alterations could be appropriate biomarkers for identifying patients eligible for targeted treatments in various medical specialties, including neurology. Targeted treatments are already approved for certain neurological diseases such as DMD, SMA and FAP and analysis of the relevant gene mutations is mandatory and should be considered predictive biomarker for treatment selection. Furthermore, several clinical trials are also aiming to expand the use of gene-informed therapy selection, while several targeted treatments with the associated predictive biomarkers are expected to emerge in the near future **Tab. 7. and Tab. 8.**

Antisense oligonucleotides (ASOs) are small DNA

Tab. 7. List of genes with and the associated diseases that could lead to modification of patients' management.

Phenotype	GENE	Type of intervention	Therapeutic intervention	References
Dopa-responsive dystonia	GCH1, TH, SPR, PTPS	Medication	Levodopa	(109)
Dystonia	TOR1A (DYT1), KMT2B (DYT28),	Medication, DBS	No response to levodopa, good response to anticholinergics, good DBS candidates	(110)
Dystonia	THAP1 (DYT6)	Medication, DBS	No response to levodopa, good response to anticholinergics, variable DBS response	(110)
Dystonia	KCTD17 (DYT 26), GNAL (DYT25)	DBS	No response to medication, good DBS candidates	(111,112)
Dystonia	ATP1A3 (DYT 12)	No effective	No response to medication, not proved response to DBS	(111,112)
Parkinson's disease	SNCA duplication, triplication	Medication effectiveness prediction, DBS	Good response to levodopa iniatially, maybe worse later, small series of DBS patients-possible good candidates	(111,112)
Parkinson's disease	SNCA missense	Medication effectiveness prediction	Good response to levodopa iniatially, maybe worse later, poor response to DBS	(111,112)
Parkinson's disease	LRRK2, PINK1	Medication effectiveness prediction, DBS	Good response to levodopa, good DBS candidates	(111,112)
Parkinson's disease	VPS35	Medication effectiveness prediction, DBS	Good response to levodopa, small series of DBS patients-possible good candidates	(111,112)
Parkinson's disease	Parkin (PRKN)	Medication effectiveness prediction, DBS	Good response to levodopa (frequent motor complications), excellent DBS candidates	(111,112)
Parkinson's disease	DJ1 (PARK7)	Medication effectiveness prediction	About 50% of patients respond effectively to levodopa, not proved response to DBS	(111,112)
Episodic Ataxia Type 1	KCNA1	Medication choice	Carbamzepine/ acetazolamide / phenytoine/ valproic acid/ lamotrigine	(113,114)
Episodic ataxia Type 2	CACNA1A	Medication choice	Acetazolamide/ 4-aminopyridine Dalfampridine/ Levetiracetam (in combination with acetazolamide)	(115)

Paroxysmal exercise induced dyskinesias/ epilepsy	SLC2A1	Diet recommendations	ketogenic diet	(115)
Vitamin B6-deficient epilepsy	ADH7A1	Diet recommendation	Pyroxine, lysine-restricted diet	(115)
Developmental and epileptic encephalopathy	CAD	Diet recommendation	Uridine	(115)
Ataxia and refractory myoclonic epilepsy	Folate cycle genes: FOLR-1, MTHFR, DHFR, PCFT	Diet recommendation	Folinic acid, 5-methyltetrahydrofolate	(115)
Vitamin B6 deficient epilepsy	PNPO, PLPBP	Diet recommendation	Pyridoxal-5-phosphate, Pyrodoxine	(115)
Vitamin B6 deficient epilepsy	PNPO	Diet recommendation	Pyridoxal-5-phosphate	(115,116)
Epileptic encephalopathy	PIGA	Diet recommendation	Ketogenic diet	(115)
Dravet syndrome	SCN1A	Medication recommendation	Valproic acid (VPA) +/- Clobazam Stiripentol Topiramate Fenfluramine Cannabidiol Bromide Avoidance of sodium channel blockers	(115)
Ohtahara syndrome, early encephalopathy/ Developmental and epileptic encephalopathy	SCN2A/SCN8A	Medication recommendation	Sodium channel blockers	(115)
Developmental and epileptic encephalopathy 12	PLCB1	Medication recommendation	Inositol	(115)
Developmental and epileptic encephalopathy	KCNA2	Medication recommendation	4-Aminopyridine	(115)
Developmental and epileptic encephalopathy	KCNQ2	Medication recommendation	Sodium channel blockers , Retigabine, Gabapentin	(117)
Epilepsy of infancy with migrating focal seizures	KCNT1	Medication recommendation	Quinidine	(118)
Early-onset epileptic encephalopathy	GRIN2A	Medication recommendation	Memantine, Dextromethorphan for gain of function variants	(119)
PRRT2-related infantile seizures	PRRT2	Medication recommendation	Carbamazepine and Oxcarbazepine	(120)
TSC-associated focal seizures	TSC1/2	Medication recommendation	Everolimus, Sirolimus, Rapamycin	(121)
Duchenne/ Becker (DMD)	Dystrophin	Targeted therapy	Eteplirsen, Golodirsen, Ataluren	-
Spinal Muscular Atrophy	SMN2	Targeted therapy	Nusinersen	(121)
Spinal Muscular Atrophy	SMN1	Targeted therapy	Onasemnogene abeparvovec	-
RPE65-mediated Inherited Retinal Dystrophy	RPE65	Targeted therapy	Voretigene Neparvovec	(122,123)
(DBS: Deep Brain Stimulation)				

sequences, effective in neutralizing defective or harmful gene products, since they can suppress the expression of a target gene at the post-transcriptional phase. Advances in their design and chemical properties have allowed safe and effective delivery to the central nervous system. The successful implementation of ASOs therapy against SMN1/2 in spinal muscular atrophy (SMA), paved the way for their utilization in other diseases, such as ALS. Over the past two decades, ASOs treatments for ALS have evolved significantly. An ASOs treatment has recently been approved for superoxide dismutase 1 (SOD1) ALS, while ASOs targeting C9orf72, FUS, and ATXN2 are under investigation in clinical trials for familial or sporadic forms of the disease [109,110]. Moreover, an exciting opportunity for CRISPR/Cas9-mediated gene therapy targeting repeat expansion mutations has also emerged. CRISPR gene-editing machinery transported by adeno-associated viruses can excise the expansions and

Tab. 8. Gene informed clinical trials in neurology (accessed on 6/07/2022).

Gene	Clinical trial	Phase	Intervention	Location
ALS/FTD				
SOD1	NCT04856982	3	BIIB067 (Tofersen)	USA
SOD1	NCT04744532	1	Bosutinib	Japan
FUS	NCT04768972	3	ION363	
C9orf72	NCT04993755	2	TPN-101	USA
C9orf72	NCT04931862	1/2	WVE-004	Australia
C9orf72	NCT03987295	2	AL001	USA
C9orf72	NCT04220021	2	Metformin	USA
ATXN2	NCT04494256	1	BIIB105	USA
Huntington				
IT15	NCT05243017	1/2	AMT-130	Europe
IT15	NCT04120493	1/2	AMT-130	USA
Transthyretin-Related (ATTR) Familial Amyloid Polyneuropathy				
TTR	NCT04601051	1	NTLA-2001	Europe
TTR	NCT05071300	3	Eplontersen	USA
Parkinson				
GBA	NCT05287503	2	Ambroxol Hydrochloride	Europe
GBA	NCT04127578	1/2	LY3884961	USA
LRRK2	NCT05418673	3	BIIB122	USA
Gaucher				
GBA		4	Cerezyme® / Imiglucerase	China
GBA	NCT03485677	3	Eliglustat (GZ385660)/Imiglucerase	USA/Canada
GBA	NCT04411654	1/2	LY3884961/ Methylprednisolone/ Sirolimus/ Prednisone	USA
Alzheimer's Disease				
APP	NCT05269394	2/3	E2814/ Lecanemab	USA
APP	NCT01760005	2/3	Gantenerumab, Solanezumab	USA
APOE4	NCT05400330	1	LX1001	USA
APOE4	NCT03634007	1	LX1001	USA
APOE4	NCT04770220	3	ALZ-801	USA
Epilepsies				
SCN1A (Dravet syndrome)	NCT05419492	1/2	ETX101	Not yet recruiting
SCN8A-DEE	NCT05226780	2	NBI-921352	Not yet recruiting
SCN8A-DEE	NCT04873869	2	NBI-921352	USA
KCNQ2	NCT04639310	3	XEN496	USA
KCNQ2	NCT04912856	3	NBI-921352	USA
Hereditary Retinal Dystrophies				
ND4	NCT04912843	1/2	NR082	China
ND4/11778 and ND1/3460	NCT04561466	2/3	Béfizal	France
CEP290	NCT04855045	2/3	sepofarsen	Several locations
USH2A	NCT05158296	2/3	QR-421a	USA
RPGR	NCT04850118	2/3	AGTC-501 rAAV2tYF-GRK1-hRPGRco	USA
RLBP1	NCT03374657	1/2	CPK850	Sweden
PDE6A	NCT04611503	1/2	rAAV.hPDE6A	Germany
RPGR	NCT03316560	1/2	rAAV2tYF-GRK1-RPGR	USA
RPGR	NCT04517149	1/2	4D-125 IVT Injection	USA
RPGR	NCT04671433	3	AAV5-RPGR	USA
Neuropathies				
Gigaxonin	NCT02362438	1	scAAv9/JeT-GAN	USA
SORD	NCT05397665	2/3	AT-007	USA
Spastic Paraplegia				
PCSK9	NCT04101643	1/2	evolocumab	China
AMN	NCT05394064	1/2	SBT101	USA
Charcot Marie Tooth				
PMP22 dup	NCT05092841	3	PXT3003	USA

PMP22	NCT05333406	1	EN001	Korea
Duchenne Muscular Dystrophy				
DMD	NCT05429372	2	PF-06939926	Not yet recruiting
DMD	NCT04004065	2	SRP-5051	USA
DMD	NCT05096221	3	SRP-9001	USA
DMD	NCT03992430	3	Eteplirsen	USA
DMD	NCT02500381	3	SRP- 4045/ SRP- 4053	USA
DMD	NCT04336826	2	Ataluren	USA
Spinal Muscular Atrophy				
SMN1	NCT05335876	2	onasemnogene abeparvovec (Zolgensma)	USA
SMN2	NCT05115110	2/3	RO7204239/Risdiplam	Belgium
SMN1	NCT05386680	3	OAV101	Not yet recruiting
SMN1	NCT04851873	3	OAV101	USA
SMN1	NCT05089656	3	OAV101	Several locations
SMN2	NCT04089566	2/3	Nusinersen	USA
SMN2	NCT04488133	4	Nusinersen	USA

eliminate disease induced pathology. For example, the effective deletion of the hexanucleotide repeat expansion mutations in the C9ORF72 locus is expected to reduce pathological hallmarks of C9ORF72 ALS/FTD. Hence, excising C9orf72 expansions by the CRISPR/Cas9 genome editing has been proposed as a possible treatment strategy to eliminate the disease pathology [111,112]

Huntington's disease is also another paradigm of disease that targeted treatments have been tested based on the knowledge of its genetic cause. Recent research focused on HTT/mHTT-lowering strategies using ASOs. Although some ASOs have failed in late-stage trials, new treatments continue to be studied. The use of ASOs or CRISPR-Cas9 presents a promising field of research for the treatment of this disorder, as well as for many other repeat expansion disorders.

For Parkinson's disease, the development of the therapeutic strategy has also focused on the most common genetically linked targets alpha-synuclein (SNCA), leucine-rich repeat kinase-2 (LRRK2) and glucocerebrosidase (GBA1) [113,114]. LRRK2 mutations are the most common cause of autosomal dominant PD accounting for 5–15% of dominant familial PD and 1–3% of sporadic PD. LRRK2 is a viable drug target in both monogenic and sporadic PD. It has been shown that LRRK2 inhibition has the potential to correct lysosomal dysfunction in patients with PD at doses that are generally safe and well tolerated, warranting further clinical development of LRRK2 inhibitors as a therapeutic modality for PD [115].

Targeted molecular therapies have also been tested in Alzheimer's disease using three disease-related genes as potential targets: Amyloid precursor protein (APP), Microtubule-associated tau protein (MAPT) and Apolipoprotein E (APOE) [116,117]. For example, several *APP* mutations increase the risk of early-onset Alzheimer's development. However, there is also one mutation, the A673T, that prevents disease development by reducing the cleavage of APP by β-secretase. It has been proposed that the insertion of this protective mutation in patients' neurons *in vivo* could prevent hereditary AD and eventually also sporadic AD [118,119]

CONCLUSION

Technological advances have allowed the development of accurate and comprehensive genetic analysis in several medical fields including Neurology. The applicability and utility of the new sequencing technologies are also dependent on the interdisciplinary collaboration to guide each patient towards the most appropriate for his phenotype test, with the right technology at an affordable analysis cost. Importantly, NGS based genomic analysis can provide a valuable diagnostic tool for heterogeneous disorders, resolving a diagnostically vague picture and providing a definitive and concise diagnosis that is indispensable for the appropriate management of the patient.

In the past, genetic analysis for hereditary neurological conditions was rarely requested and performed mainly for diagnostic purposes using single gene analysis methodologies. The evolution of new technologies led to increased diagnostic rates for several morbidities and provided insights about their utility for the management of the patients and their relatives [120-127]. The expanded application of advanced NGS technologies is informing new gene therapies clinical trials and is constantly increasing our knowledge concerning the genetic component of several neurological diseases. Thus, precision medicine and the application of gene-informed targeted treatments is expected to become a reality soon. Consequently, the information received from genetic analysis, especially from a comprehensive NGS analysis such as WES and WGS is valuable not only for diagnosis and management of the patients' current condition but in addition is a dower for the future upcoming gene-directed treatments.

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AVAILABILITY OF DATA AND MATERIALS

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EP performed the literature search and writing of this manuscript. GP performed literature search and contributed to writing the manuscript. SK offered scientific advice, reviewed & performed major editing of the manuscript. MC offered consultation, contributed to literature search about clinical utility of the genetic analysis in Neurology and performed editing. NG offered expert scientific advice, reviewed & performed major editing of the manuscript. VK offered scientific advice, reviewed & performed major editing of the manuscript. GT provided scientific advice, reviewed, and edited the final manuscript. DM offered advice and corrected the manuscript final version. EC offered consultation and performed major review and editing of the manuscript. ED performed major reviews and editing. GT performed the analysis of the clinvar data. GN contributed to the writing and literature search.

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