How have advances in genetic technology modified movement disorder nosology?

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The role of genetics and its technological development have been fundamental in advancing the field of movement disorders, opening the door to precision medicine. Starting from the revolutionary discovery of the locus of the Huntington's disease gene, we review the milestones of genetic discoveries in movement disorders and their impact on clinical practice and research efforts. Before the 1980s, early techniques did not allow the identification of genetic alteration in complex diseases. Further advances increasingly defined a large number of pathogenic genetic alterations. Moreover, these techniques allowed epigenomic, transcriptomic and microbiome analyses. In the 2020s, these new technologies are poised to displace phenotype-based classifications towards a nosology based on genetic/biological data. Advances in genetic technologies are engineering a reversal of the phenotype-to-genotype order of nosology development, replacing convergent clinicopathological disease models with the genotypic divergence required for future precision medicine applications.

Introduction

"*The success of any genetic linkage project depends in large part on the quality of family material available for the study. We initially invested considerable effort in identify a useful family [. . .]*" [1]. This statement, extracted from the article presenting the discovery of the mutation responsible for Huntington's disease (HD), represents a milestone in the history of medicine, i.e. the first genetic disease mapped without any *a-priori* knowledge of its localization [1,2]. This event represented a watershed in the genetics of movement disorders between the first era, characterized by a few successes, and a new one marked by an ever-increasing number of genetic discoveries.

This quote, although accurate, encompasses the old paradigm in the approach to genetic analysis, based on the flow of information from phenotype to genotype where a comprehensive assessment and description of family history were essential to generate the genetic causal hypotheses needed to identify the genetic abnormality responsible for the observed phenotype. This approach reflected the technological limitations in the DNA analysis at that time, for which a clear mode of genetic inheritance and evidence for a monogenic disease trait were necessary, limiting the knowledge to rare or low-penetrance conditions.

Toward the end of the first decade of this century, as the initial phase of the Human Genome Project concluded [3] and with breakthrough advances in DNA sequencing technologies, a 'blind' analysis of a single patient's DNA became possible. In this era, the flow of information was reversed, i.e. genotypes were obtained before phenotypes and our approach to proving a causal genetic hypothesis was also inverted. This technological development came with advances in the knowledge of the complexity of genetic interactions, such as the impact of regulatory elements on coding variants [4] or the stochastic effect of aging on

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somatic mutations [5], adding challenges to the interpretation of novel genetic findings.

We here review the milestones of genetic discoveries in movement disorders and how these altered the clinical management of patients and the design of clinical and research programs. We also draw attention to the interface of biomarker discovery programs, ushering in the era of 'precision medicine' for movement disorders [6,7].

Before 17 November 1983: cytogenetic and HLA analysis

After the major discovery of the 20th century, namely the description of human DNA by Watson and Crick in 1953 [8], genetic studies emerged as a crucial component in scientific research. However, it was not until 1956 that the number and structure of human chromosomes were first described [9]. Soon after these two discoveries, Down's syndrome became the first disease to be recognized as associated with aberrant chromosomes [10]. However, cytogenetics was still inadequate to detect causal genetic factors in movement disorders, leading to limited applicability. For the first and limited applicability in movement disorders, we had to wait until the discovery of the physical localization of the human leukocyte antigen system (HLA) loci on chromosome 6 and, with it, to serve as a map for genomic location [11,12] using linkage analyses, intragenic recombination and chromatography techniques [13,14]. HLA linkage analysis first suggested that the spinocerebellar ataxia (SCA)1 gene locus might be located on chromosome 6 near the HLA loci, based on studies of a family of five patients with the previously named Marie's ataxia [15,16]. Later on, in 1977, Jackson et al. [17] refined the location of the SCA1 locus on chromosome 6 at a 12-cM distance from the HLA loci.

However, the possibility of obtaining information related only to the HLA locus limited our knowledge for the vast majority of diseases. Subsequently, in the 1980s, further improvements in gene mapping were based on restriction fragment length polymorphisms, which allowed the analysis of the whole genome [18].

After 17 November 1983: mapping of Huntington's disease gene

This day marked the beginning of a revolution for the medical field, i.e. the first gene was mapped analyzing the entire DNA without *a-priori* knowledge of its physical localization [1,2]. Since the 1970s, HD had been considered a neurodegenerative autosomal dominant genetic disorder [19]. Blood group A was initially found to be associated with HD [20] but large population studies failed to confirm this association. At that time, neither gross morphology nor alteration of number of chromosomes had been detected in HD [21]. With the advent of restriction fragment length polymorphism analysis, it finally became possible to identify the specific HD locus. This result gave a significant boost to genetics, allowing the discovery of further loci responsible for other conditions, such as SCA1 in 1987 [22], Friedreich's ataxia in 1988 [23] and early-onset generalized dystonia (DYT1) in 1989 [24]. However, several years elapsed before further technical developments facilitated the identification of the specific mutation responsible for each of these conditions. This step was taken thanks to the possibility of capturing and amplifying specific regions of the genome by the polymerase chain reaction technique in the 1990s [25] together with advances in Sanger sequencing [26]. In 1993, the exact mutations responsible for HD and SCA1 [27,28] were identified, followed by many other such successes, such as for Friedreich's ataxia in 1996 [29] and DYT1 in 1997 [30].

A special mention should be made of the identification of the genetic mutation responsible for an autosomal dominant form of Parkinson's disease (PD), namely *SNCA* (PARK1). The first localization of this gene on chromosome 4 (4q21.3-q22) was documented by Chen et al. in 1995 [31]. At the time, it was called the non-amyloid component of Alzheimer's disease plaques, given that it was the second most common component found in the amyloid plaques of Alzheimer's disease pathology [32]. In 2 years, the first genetic variant (Ala to Thr substitution at position 53, Ala53Thr) was found in the 'Contursi family' [33]. In the same year (1997), Spillantini and coworkers [34] found the expression of the protein coded by this gene as the molecule constituting Lewy bodies in patients with PD and dementia with Lewy bodies. Notably, a causal implication was readily given to these findings, as stated by Spillantini and coworkers in their original publication: "*α-Synuclein aggregation and Lewy-body formation may be important in the aetiology and pathogenesis of all cases of Parkinson's disease.*" The demonstration of the first genetic form of PD was interpreted as informing the whole of the clinico-pathological construct of PD. Separately in the same year, Japanese investigators identified the Parkin gene as a genetic variant responsible for a recessive form of PD (*PRKN*, *PARK6*) [35]. Interestingly, some of the mutation-positive patients had been previously shown to have no Lewy body pathology on autopsy [35-37]. If the Contursi kindred had carried *PRKN* instead of *SNCA*, there might not have been a pathogenic premise for α-synuclein and Spillantini's discovery would

not have framed pathology as equating etiology. Nevertheless, the pathogenic centrality of α-synuclein became enshrined in most subsequent research efforts.

Although the expectation was that discoveries of genetic variants in parkinsonisms and dystonia, for instance, might inform therapeutic management, there have been no disease-modifying treatments established to date. Some genetic variants have been associated with different outcomes in device-aided therapies, such as deep-brain stimulation (DBS) [38]. For example, a recent meta-analysis showed a higher post-DBS reduction in levodopa-equivalent daily dose in *LRRK2*- and *PRKN*-associated parkinsonism (around 60%) compared with *GBA*-associated parkinsonism (around 20%) [38]. Conversely, *GBA*-mutated patients demonstrated a higher rate of cognitive impairment after DBS compared with other genetic parkinsonisms [38]. Differential clinical responses to DBS have also been ascertained for dystonia, where, for example, response is more favorable in *TOR1A* compared with other variants [39].

Many other discoveries were made in movement disorders in the 1990s and 2000s (Tables 1–4; Fig. 1) [28,40-62]. The approach during this period was disease centered rather than patient centered; data on many patients with the same conditions and within the same families were necessary to describe the possible loci of disease-related genes to meet the technological needs. Finally, in the 2000s, with the advent of 'next-generation sequencing' (NGS), a personalized genetic approach became possible. A full reversal of the phenotype-to-genotype approach was finally engineered, enabling the study of a single patient, one for whom the phenotype could not inform a locus, let alone a gene.

Next-generation sequencing era

With the end of the Human Genome Project, the sequence of the entire human DNA was finally available [3]. However, the cost and time required to sequence the entire genome were limiting steps for clinical applicability [63]. With NGS, a term that refers to a number of different techniques capable of analyzing fragments of DNA in parallel [64], these two major limitations have been, at least partially, overcome. In 2008, the genome of the DNA pioneer James Watson was the first to be sequenced with NGS [65] with a significant reduction in cost (1% of the cost) and time (4.5 months vs. 4 years) [66]. NGS was not only important in discovering new gene variants associated with movement disorders [67] but was also fundamental in revisiting genetic conditions that were already known (Fig. 2). An emblematic example

is the paroxysmal disorders (Table 4), for a long period classified exclusively as channelopathies and now considered as a more complex spectrum of genetic diseases [68]. The discovery of new genes not coding for ion channels shifted the pathophysiological understanding of these conditions and has opened the door to novel therapeutic opportunities [68]. For instance, the identification of mutations in *SLC2A1* that affect the glucose type-1 transporter, prompting consideration of a ketogenic diet for the individualized therapeutic approach to these patients [68].

Another advantage of NGS is the possibility of combination with previous techniques, such as linkage analysis, which can be a useful approach for the analysis of genetic conditions with a clear familial predisposition. In these cases, we can use linkage analysis and select only a few regions that can subsequently be analyzed with NGS, thus reducing the coverage required for the analysis. An important success using this combination was the discovery of *COQ2* variants in multiple system atrophy [69].

Importantly, NGS techniques have extended their reach beyond the genome, becoming fundamental in the analysis of other biological data, such as epigenetic alterations of the DNA, the transcriptome (analysis of the mRNA) and the characterization of metagenomes [70]. The possibility of deeply analyzing the complexity of biological interactions directly challenges clinical-based disease classifications allowing the advent of an era in which the biological data represent the 'gold standard' for reassessing clinical nosology and disease-modification efforts in complex diseases [71]. Indeed, the traditional genetic classification of diseases into polygenic and monogenic is under revision [72]as the effects of the regulatory variants can modulate the penetrance of coding variants [73]. Therefore, a reconstruction process in genetic movement disorders should treat as independent variables those genomic variants with larger effects (i.e. the classical disease-causing mutations in genes such as *GBA* or *LRRK2*) [74,75] and as co-variates a vast number of factors acting as penetrance modifiers. Regulatory variants have been shown to be important in neurodevelopmental processes, affecting not only the penetrance but also the clinical presentation of several conditions [76]. Moreover, patients harboring more than one gene mutation in certain monogenic parkinsonisms have been described, adding layers of complexity to the diagnosis and future treatment approaches [77].

Some important genetic variants have been validated as risk factors for movement disorders. For instance, the *GBA* p.E365K variant has been associated with a higher risk of cognitive impairment and

Table 1 Preferred genetic approach for selected genetic parkinsonian disorders

Name and modality of inheritance	Year of first reported mutation	Suggested genetic diagnostic approach	Key clinical features
SNCA (PARK1, PARK4) AD	1997	Single gene analysis (Sanger Seq) or multigene panel	Early-onset levodopa-responsive parkinsonism, cognitive, behavioral and/or autonomic symptoms, myoclonus
PRKN (PARK2) AR	1997	Single gene analysis (Sanger Seq and MLPA) or multigene panel and MLPA	Early-onset levodopa-responsive parkinsonism; low risk for cognitive impairment
PINK1 (PARK6) AR	2004	Multigene panel or WES	Early-onset levodopa-responsive parkinsonism; possible cognitive and/or behavioral manifestations
DJ-1 (PARK7) AR	2003	Multigene panel or WES	Rare, early-onset levodopa-responsive parkinsonism; low risk for cognitive manifestations
LRRK2 (PARK8) AD	2004	Single gene analysis (Sanger Seq) or multigene panel	Sporadic PD-like AAO, levodopa-responsive parkinsonism with less incidence of cognitive decline. Highly variable pathology
ATP13A2 (PARK9) AR	2006	Multigene panel or WES	Rare, juvenile-onset levodopa-responsive dystonia and atypical parkinsonism; possible eye-movement abnormalities and pyramidal signs. Wide clinical spectrum ranging from parkinsonism to hereditary spastic paraplegia
FBXO7 (PARK15) AR	2008	Multigene panel or WES	Rare, spasticity followed by juvenile-onset levodopa-responsive atypical parkinsonism
VPS35 (PARK17) AD	2011	Multigene panel or WES	Rare, early-onset levodopa-responsive parkinsonism with possible cognitive and/or behavioral symptoms
DNAJC6 (PARK19) AR	2012	Multigene panel or WES	Rare, juvenile atypical parkinsonism with cognitive impairment, epilepsy and pyramidal signs; high phenotypic variability
SYNJ1 (PARK20) AR	2013	Multigene panel or WES	Rare, juvenile atypical parkinsonism; cognitive impairment and/or seizures
VPS13C AR	2016	Multigene panel or WES	Rare, early-onset atypical parkinsonism with rapid progression and early cognitive impairment

This table was drafted according to the following references [40-42]. AAO, age at onset; AD, autosomal dominant; AR, autosomal recessive; MLPA, multiplex ligation-dependent probe amplification; PD, Parkinson's disease; WES, whole-exome sequencing.

rapid eye movement sleep behavior disorders in patients with PD, whereas other variants of the same gene, such as p.N370S and p.T408M, have been associated with higher motor disability [78]. Many other genetic variants have been associated to varying degrees with the development of PD, such as *TMEM230*, which encodes a transmembrane protein involved in vesicle trafficking [79], *SLC6A3*, which encodes human dopamine transporter gene [80] or catechol-O-methyltransferase haplotypes [81]. In dystonias, the rs1801968 *TOR1A* variant increases the risk of a focal phenotype (writer's cramp) [82]. Furthermore, selected variants in genetic variants associated with an increased risk of PD are opening the possibility of exploring lysosomal (*GUSB*, *GRN* and *NEU1*) and endocytic (*VAMP4* and *NOD2*) pathways [83].

The analysis of single-nucleotide polymorphisms could help future therapeutic developments in suitable patients using strategies based on drug repurposing [84]. Large cohorts and data-driven analyses will assist in identifying genetic and/or biological alterations in a range of phenotypes, helping us transition from a phenotypic-based classification of diseases to a biologically driven one.

Influence on biomarker development programs for neurodegenerative diseases

The century-old view of neurodegenerative diseases as single if complex entities has been useful for the development of symptomatic treatments to compensate for the deficiency of neurotransmitters resulting from

Table 2 Preferred genetic approach for selected genetic dystonias

Name and modality of inheritance	Year of first reported mutation	Suggested genetic diagnostic approach	Chief clinical features
TOR1A (DYT1) AD	1997	Single mutation analysis or multigene panel	Childhood or adolescent-onset generalized dystonia usually starting in one limb (leg), without other systemic involvement (isolated). Most common form
TAF1 (DYT3) XR	2007	WGS	Adult-onset dystonia characterized by dystonia and parkinsonism with associated neurodegeneration. Endemic in the Philippines
TUBB4 (DYT4) AD	2013	Multigene panel or WES	Childhood-onset cranio-cervical dystonia with prominent spasmodic dysphonia ('whispering dysphonia'). May include leukoencephalopathy
GCH1 (DYT5a) AD	1994	Multigene panel or WES	Childhood-onset (in general onset at first decade) dopa-responsive dystonia. Lower body dystonia with gait impairment and development of parkinsonian features. Sleep and mood disorders as non-motor features
TH (DYT5b) AR	1995	Multigene panel or WES	Infant-onset dopa-responsive dystonia. Lower limb onset with gait difficulties. Fluctuations of symptoms (better in the morning and worse in the evening). Bradykinesia, hypotonia and autonomic can be present
SPR (DYT5b) AR	2001	Multigene panel or WES	Childhood-onset partially dopa-responsive dystonia with motor and speech delay. Hypotonia, intellectual disability, psychiatric symptoms, autonomic dysfunction and sleep disturbance can be present
THAP1 (DYT6) AD	2009	Multigene panel or WES	Adolescent-onset dystonia of mixed type, mainly affecting cranial, cervical and laryngeal areas. It may be generalized
SGCE (DYT11) AD	2001	Single gene analysis (Sanger Seq) or multigene panel or WES	Childhood-onset myoclonus-dystonia, affecting neck, upper limb and trunk. Possible association with psychiatric symptoms (Klein, 2014; Klein *et al.*, 2017)
PRKRA (DYT16) AR	2008	Multigene panel or WES	Infancy/childhood-onset dystonia, in particular oromandibular, associated with parkinsonism, dysphagia and retrocollis. No response to levodopa
ANO3 (DYT24) AD	2012	Multigene panel or WES	Adult-onset focal or segmental dystonia with predominantly cranial-cervical dystonia
GNAL (DYT25) AD	2013	Multigene panel or WES	Adult-onset segmental cranial-cervical dystonia
KMT2B (DYT28) AD	2016	Multigene panel or WES	Childhood-onset (first decade) generalized dystonia with prominent cervical, cranial and laryngeal involvement. Psychiatric symptoms, skull abnormality, skin and eye movement abnormalities

This table was drafted according to the following references [41,43-54]. AD, autosomal dominant; AR, autosomal recessive; WES, whole-exome sequencing; WGS, whole-genome sequencing; XR, X-linked recessive.

degeneration of vulnerable neurons, a convergent outcome of many processes, which is presumably different in different individuals. The characterization of the unique genetic and biological features of each patient is expected to translate into the first disease-modifying successes when therapies will be applied not to groups of clinically defined cohorts but only to individuals biologically suited to benefit, which is the basis of precision medicine [6,7]. To answer this need, some biomarker initiatives have been launched in recent years to assess the extent to which genetic and biological/molecular abnormalities may affect patterns of disease progression. Starting from 2011, many cohorts, such as the Parkinson's Progression Markers Initiative [85], Luxembourg cohort [86] and Personalized Parkinson Project [87], have been built with the aim of establishing a wide-ranging set of clinical, imaging and biological data to define biomarkers of the clinical PD progression and for possible therapeutic strategies. However, these cohorts aim at the identification of biomarkers explaining the clinical diagnosis of PD as the independent variable, with biological measures as the dependent variables.

In 2019, the University of Cincinnati launched the Cincinnati Cohort Biomarker Program with the aim of identifying biomarkers of subtypes regardless of clinical classifications (e.g. PD-like/Alzheimer's disease-like conditions) (Methods paper submitted for review). In this phenotype-agnostic cohort, the biological signals are considered as the independent variables and the clinical phenotypes as the dependent variable. The Cincinnati Cohort Biomarker Program

Table 3 Preferred genetic approach for selected genetic choreas

Name and modality of inheritance	Year of first reported mutation	Suggested genetic diagnostic approach	Chief clinical features
HTT AD	1993	Single mutation analysis (repeat expansion assesment)	Adult-onset progressive chorea associated with irritability, depression and cognitive impairment. Juvenile form (Westphal) has faster progression
ADCY5 AD	2012	Multigene panel or WES	Childhood-onset chorea with dystonia, myoclonus and delayed motor and/or language milestones. Axial hypotonia common. No response to levodopa
NKX2-1 AD	2002	Multigene panel or WES	Childhood-onset chorea with possible dysarthria, axial dystonia and gait disturbances. No response to levodopa
PDE10A AR, AD	2016	Multigene panel or WES	Childhood-onset chorea with characteristic symmetrical bilateral striatal lesions on imaging

This table was drafted according to the following references [41,55]. AD, autosomal dominant; AR, autosomal recessive; WES, whole-exome sequencing.

Table 4 Preferred genetic approach for selected paroxysmal disorders

Name and modality of inheritance	Year of first reported mutation	Suggested genetic diagnostic approach	Chief clinical features
PRRT2 AD	2009	Single gene analysis (Sanger Seq) or multigene panel or WES	Childhood-onset paroxysmal kinesigenic dyskinesia associated with epilepsy. General brief chorea/dystonia episodes, <1 min
PNKD AD	2004	Multigene panel or WES	Childhood-onset dystonic and choreic episodes with dysarthria, dysphagia, oculogyric crises; episodes last hours
SLC2A1 AD	2008	Multigene panel or WES	Variable age-onset (often in second decade) of choreic and dystonic attacks triggered by sustained exercise. Ataxia and epilepsy can be associated
ECHS1 AR	2015	Multigene panel or WES	Infant-onset paroxysmal exercise-induced dystonia. Episodes last 30–60 min with dystonia/opisthotonus. Leigh syndrome can be associated
KNCMA1 AD	2005	Multigene panel or WES	Infant-onset paroxysmal non-kinesigenic dyskinesia. Attacks are brief. Often associated with epilepsy and mental retardation

This table was drafted according to the following references [56]. AD, autosomal dominant; AR, autosomal recessive; WES, whole-exome sequencing.

is a population-based longitudinal study of patients with a wide range of neurodegenerative diseases and healthy controls to be followed for up to 10 years. Genetic and biological/molecular analyses and deployment of bioassays of already available therapies will allow the repurposing of these for patients with suitable biology.

Five-year view

Increased knowledge of the role of non-coding gene regions (e.g. due to splicing alteration) [88] is anticipated to further reshape the nosology of movement disorders. For instance, an intronic biallelic pentanucleotide expansion in chromosome 4 has recently been associated with cerebellar ataxia, neuropathy and vestibular areflexia syndrome [89]. New insights into the functional interaction between coding and non-coding regions, and the development of new technologies such as long-read NGS to identify non-coding variants, will improve the discovery of new genetic variants associated with movement disorders, which must go hand in hand with development in the computational frameworks. Non-coding region analysis is likely to become increasingly important in the regular workflows of clinicians.

Figure 1 Schematic historical perspective of genetic analysis in spinocerebellar ataxias (SCAs). NGS, next-generation sequencing; SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing. This figure was drafted according to the following references [28,57-62].
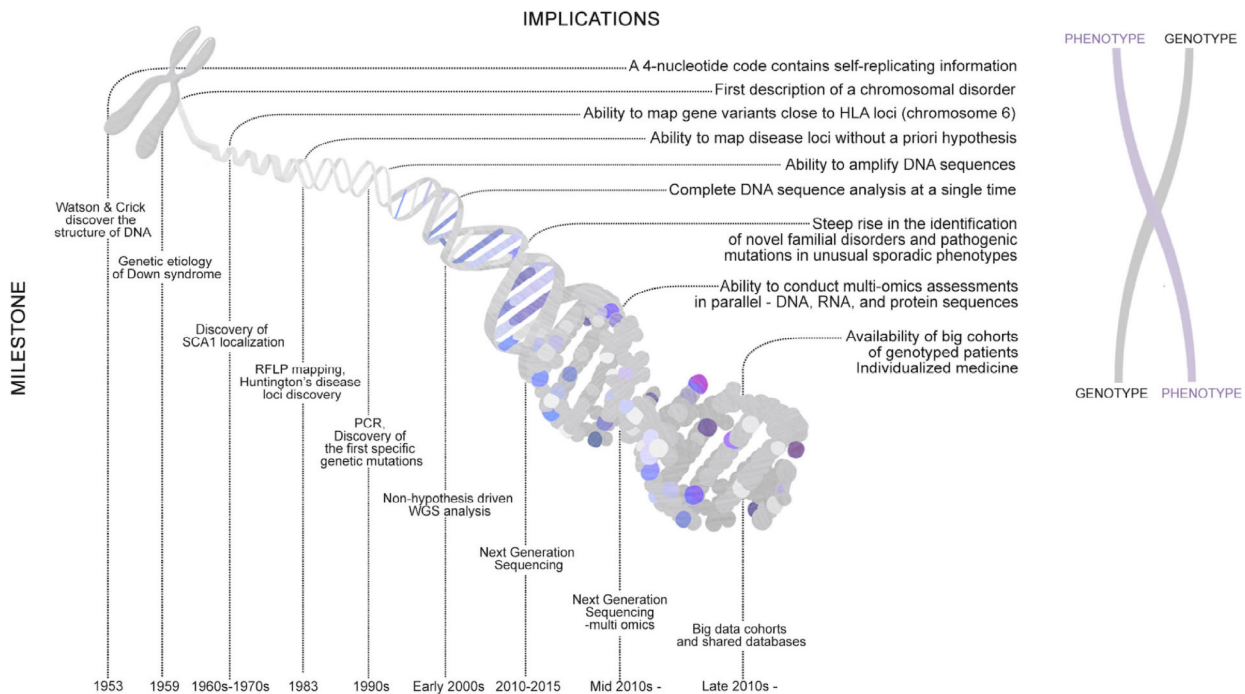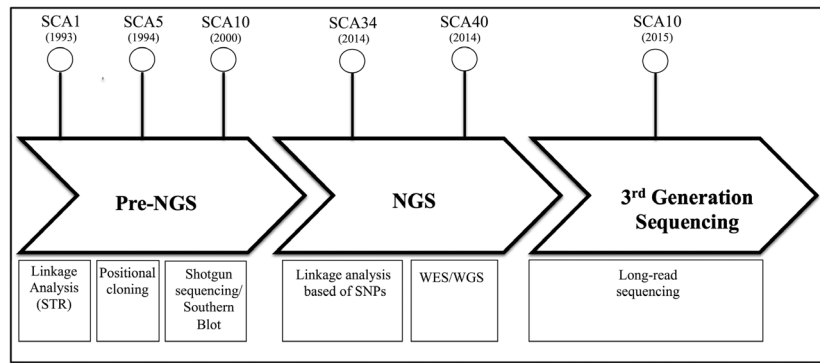
Figure 2 Milestones in genetics of movement disorders and reversal of the phenotype-to-genotype developmental order. The main discoveries in the field of movement disorders, shown on a chronological timeline, demonstrate the increasing power of the detection of pathogenic mutations in individuals with no family history in a phenotype-agnostic manner. DNA, deoxyribonucleic acid; HLA, human leukocyte antigen; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RNA, ribonucleic acid; SCA1, spinocerebellar ataxia type 1; WGS, whole-genome sequencing.

Challenges and conclusions

Despite the important revolution that new genetic technologies have ushered into the field of movement disorders, there are some obstacles to their utilization. Firstly, although costs have been reducing, NGS remains prohibitively expensive for deployment in the routine clinical setting [90]. In addition, the accuracy for identifying repeat expansions, copy number variations and structural rearrangements of short-read NGS is limited. For instance, considering that repeat expansions are frequent in SCAs [91], it is extremely important, when suspecting one of these conditions, to also test for the most frequent underlying variants (e.g. SCA1, SCA2, SCA3, SCA6, DRPLA); this approach should also be extended in sporadic SCAs [92]. At the same time, interpreting the vast amount of rare non-coding and missense variants in individual genomes can still be challenging on clinical grounds. Additionally, NGS studies can be affected by findings of unknown significance or false positives. For instance, *CACNA1B* R1389H variant was associated with the myoclonus-dystonia syndrome, but this result was not confirmed by analyzing a large multicentric

cohort of myoclonus-dystonia cases [93]. The creation of shared large databases could accelerate and facilitate the increase of knowledge on the role played by these variants. Furthermore, a number of underpowered candidate-based gene association studies conducted over the last few decades have yielded a number of false-positive findings on which corrections continue to be necessary [94]. The development of better-designed genome-wide asoociation study (GWAS) studies during the last decade has assisted the understanding of the complex genetics of movement disorders.

In sum, the advances in genetic technologies have altered the approach to classifying and, to a lesser extent, treating patients with movement disorders. From the earlier primacy of the phenotype-to-genotype paradigm, fueled by a convergent clinicopathological model of disease, newer technologies are moving us closer to a reversed genotype-to-phenotype sequence. This realignment has the potential to usher in a systems biology model to bring the first success in precision medicine for patients with movement disorders.

Disclosure of conflicts of interest

Data availability statement

No data sharing is applicable given that no new data were created or analyzed for this narrative review.

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