

Evidence and practices of the use of next generation sequencing in patients with undiagnosed autosomal dominant cerebellar ataxias: a review

Evidências e práticas do uso do sequenciamento de nova geração em pacientes com ataxias cerebelares autossômicas dominantes não diagnosticadas: uma revisão

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ABSTRACT

Autosomal dominant cerebellar ataxias (ADCA) are heterogeneous diseases with a highly variable phenotype and genotype. They can be divided into episodic ataxia and spinocerebellar ataxia (SCA); the latter is considered the prototype of the ADCA. Most of the ADCA are caused by polyglutamine expansions, mainly SCA 1, 2, 3, 6, 7, 17 and Dentatorubral-pallidoluysian atrophy (DRPLA). However, 30% of patients remain undiagnosed after testing for these most common SCA. Recently, several studies have demonstrated that the new generation of sequencing methods are useful for the diagnose of these patients. This review focus on searching evidence on the literature, its usefulness in clinical practice and future perspectives.

Keywords: next generation sequencing; autosomal dominant cerebellar ataxias; spinocerebellar ataxias.

RESUMO

As ataxias cerebelares autossômicas dominantes (ACAD) são doenças heterogêneas com fenótipo e genótipo altamente variáveis. Podem ser divididas em ataxia episódica e ataxia espinocerebelar (SCA), sendo este último considerado o protótipo do ACAD. A maior parte das ACAD são causadas por expansões de poliglutaminas, principalmente SCA 1, 2, 3, 6, 7, 17 e atrofia dentatorubro-palidoluisiana (DRPLA). No entanto, 30% dos pacientes permanecem sem diagnóstico após o teste para essas SCA mais comuns. Recentemente, vários estudos têm demonstrado que a nova geração de métodos de sequenciamento são ferramentas úteis para o diagnóstico desses pacientes. Esta é uma revisão sistemática da literatura, com foco em sua utilidade na prática clínica e em perspectivas futuras.

Palavras-chave: sequenciamento de nova geração; ataxias cerebelares autossômicas dominantes; ataxias espinocerebelares.

INTRODUCTION






Autosomal dominant cerebellar ataxias (ADCA) comprise a group of inherited cerebellar ataxias that are clinically and genetically heterogeneous¹. They can be caused by several mechanisms, such as expansion of short tandem repeats (STR), mainly trinucleotide repeat expansions (TRE), and less commonly, single nucleotide variation and short insertions and deletions (indels). The most studied mechanism related to TRE is the one caused by expanded

polyglutamine. These proteins have a pathological gain of function with a subsequent neuronal toxic effect. In these cases, there may be an anticipation phenomenon, which is characterized by increasingly early onset of symptoms as the disease is transmitted from one generation to the next. Clinically ADCA are progressive neurodegenerative diseases that share cerebellar ataxia as the core symptom, associated with progressive cerebellar atrophy. However, other brain regions, such as the brainstem, may also be involved. Within this group, we will herein emphasize the

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spinocerebellar ataxias (SCA), often used as a synonym for autosomal dominant ataxias.

The SCA has a wide range of neurological symptoms, including gait and appendicular ataxia, dysarthria, oculomotor abnormalities of cerebellar and supranuclear origin, retinopathy, optic atrophy, spasticity, extrapyramidal, peripheral neuropathy, sphincter disorders, cognitive changes, and epilepsy^{1,2,3}. Clinical diagnosis is challenging due to large phenotypic and genotypic variability. To facilitate clinical evaluation, Harding et al. suggested a classification into three subtypes: ADCA type 1, characterized by cerebellar ataxia, optic atrophy, ophthalmoplegia, extrapyramidal symptoms, pyramidal signs, peripheral neuropathy, amyotrophy and dementia; ADCA type 2, when CA is associated with retinal degeneration; ADCA type 3, composed of “pure” cerebellar ataxias⁴ (Table 1). Currently, the classification of SCA is based on the identified mutation/expansion, also known as clinical-genetic classification⁵. Forty-eight SCA subtypes have been described to date^{6,7,8} and this number tends to grow in the following years, thanks to the availability of new DNA sequencing techniques. Table 2, adapted from a review from Sullivan et al.⁶, shows the main clinical characteristics of each SCA subtype.

In this review, we will address the challenges in diagnosis of spinocerebellar ataxias, the recent diagnostic tools that are helpful when we have a patient with negative DNA test (herein called “negative ataxias”) and future perspectives in the field.

EPIDEMIOLOGICAL CONTEXT

The prevalence of hereditary ataxias in general has been little studied. A meta-analysis by Ruano et al.⁹, which included 22 studies from 16 countries with more than 14,500 patients, showed that the average prevalence of ADCA is 2.7/100,000. However, it is worth mentioning that this prevalence is variable in different regions. International studies have been conducted to assess the prevalence of ADCA around the world. A prevalence of 3/100,000 cases was found in the Netherlands¹⁰, 4.2/100,000 in Southern Norway¹¹ and 5.6/100,000 in Portugal¹². In all studies, spinocerebellar ataxia type 3 (SCA3 or Machado-Joseph disease) is the most commonly mutation found.

Table 1. Autosomal dominant cerebellar ataxia clinical classification.

ADCA 1	SCA 1-4, 8, 12-14, 15, 17-22, 25, 27, 28, 31, 32, 34-37, 38, 42-44, 46-48, DRPLA, DNMT1
ADCA 2	SCA 7
ADCA 3	SCA 5, 6, 10, 11, 23, 26, 30, 37, 41, 45

Source: adapted from Sullivan et al.⁶

ADCA: autosomal dominant cerebellar ataxia; SCA: spinocerebellar ataxia; DRPLA: dentatorubral-pallidoluysian atrophy; DNMT1: DNA methyltransferase.

In Brazil it is believed that the great epidemiological variability is due to the founder effect of different geographical regions^{3,12,13,14,15,16}. Worldwide, several epidemiological studies have demonstrated a higher frequency of SCA 1 in countries such as Italy and India; SCA 2 in Mexico, Cuba, India and Canada; SCA 6 in Australia and Canada; SCA 8 in Finland, and DRPLA in Japan (Table 3)^{2,11,12,13,14,15,16,17,18,19,20}. The main subtype found in Brazil is SCA3, and cases of SCA 1, 2, 6, 7 and 10 occur less frequently; other types are considered very rare. Jardim et al.²¹ conducted a research on ADCA in Southern Brazil evaluating 66 cases of SCA. The authors concluded that the proportion of cases of SCA 3 was very high, suggesting an Azorean founding effect. The frequency of SCA 3 in the region was 1.8/100,000, versus 0.2/100,000 for other forms of autosomal dominant ataxia. Cintra et al.²² found an even higher prevalence in the region of São Paulo, 5/100,000, considered to date the highest prevalence of SCA 3 found in Brazil. In a study with 104 families with SCA, Teive et al.²³ found a high prevalence for SCA 3 (72.46%) followed by SCA 10 (11.6%). Braga-Neto et al.²⁴ evaluated 45 families from the Northeast of the country with ataxia and identified a high consanguinity rate (40.7%). In this series, a higher prevalence of recessive autosomal ataxias (33.3%) was identified compared to dominant autosomal ataxias (6.6%), in contrast to other Brazilian epidemiological studies. However, epidemiological studies from the north of the country are scarce, and further studies are needed to assess the prevalence of hereditary ataxias in other regions of the country. Table 4 shows the frequencies of SCA in the Brazilian territory^{21,22,23,25,26,27,28,29,30,31}.

Table 2. Phenotype characteristics of each spinocerebellar ataxia.

Associated clinical features	Genetic subtypes
Peripheral neuropathy	1, 2, 3, 4, 18, 25, 38, 43, 46
Pyramidal signs	1, 3, 7, 8, 10, 14, 15, 17, 35, 40, 43
Dystonia	3, 14, 17, 20, 35
Myoclonus	14
Parkinsonism	2, 3, 10, 14, 17, 19/22, 21
Tremor	12, 15, 27
Chorea	17, 27, DRPLA*
Cognitive impairment	2, 8, 13, 17, 19/22, 21, 36, 44, 48, DRPLA
Psychiatric symptoms	2, 17, 48
Ophthalmoplegia	2, 3, 28, 40
Visual impairment	7
Face/tongue fasciculation	36
Ichthyosiform plaques	34
Seizures	10, 19/22, ATN 1**
Narcolepsy	DNMT1***
Hearing loss	31, 36, DNMT1

Source: adapted from Sullivan et al.⁶

*dentatorubral-pallidoluysian atrophy; **atrophin-1; ***DNA methyltransferase.

MOLECULAR DIAGNOSTIC CHALLENGES IN ADCA

The diagnostic investigation of patients with ADCA involves PCR (polymerase chain reaction) technique, and is based on in-vitro amplification of specific regions of the DNA, allowing the detection of nucleotide expansions, which are the substrate for the most common ADCA worldwide^{9,10,14,22,25}. Approximately 30% of the patients investigated for ADCA by the conventional method (PCR) have negative results.

The absence of a diagnosis can be very frustrating for both the patient and the physician. Obtaining a diagnosis can be an important factor of psychological impact, prognosis, genetic counseling, preimplantation genetic diagnosis and family diagnosis. In addition, it may be essential for the development of specific treatments based on a better understanding of the mutation³².

When initial DNA investigation fails, the next step would be to conduct a gene-to-gene search, which is considered a time-consuming and expensive method. But nowadays, with the fantastic advance in the development of molecular

genetic techniques, with next-generation sequencing (NGS) technology, it is possible to carry out sequencing of several genes simultaneously, saving time and costs. Each NGS technique has its advantages and disadvantages, which must be weighed to choose the ideal method for the diagnosis.

GENETIC TECHNOLOGY EVOLUTION: SOLUTION OR ADDITIONAL PROBLEMS?

In 1977, Frederick Sanger and colleagues developed a DNA sequencing method based on chain-termination inhibitors. In this method, a DNA template is replicated using a primer and a DNA polymerase that incorporates dideoxynucleotides in the sequence synthesis, causing its early termination. After multiple reactions, DNA fragments of different lengths are formed and can be read by an automated apparatus, providing DNA sequencing³³.

Compared to Sanger sequencing, considered a gold standard for genetic sequencing, NGS is capable of sequencing

Table 3. Prevalence of spinocerebellar ataxias across the world.

Country	n	SCA1	SCA2	SCA3	SCA6	SCA7	SCA8	SCA10	SCA12	SCA14	SCA17	DRPLA	und.	References
Mexico	108	ND	45,4	12	ND	7,4	ND	13,9	ND	NR	2,8	ND	18	Alonso et al. ¹⁵
Portugal	199	ND	2,5	80,5	<1	1,25	1	ND	ND	<1	<1	8,5	26,5*	Coutinho et al. ¹²
Cuba	177	ND	86,8	1,2	ND	ND	NP	NP	NR	NP	ND	ND	12	Velázquez et al. 2009 ¹⁶
Italy	225	21	24	<1	<1	<1	<1	ND	ND	NP	<1	<1	41	Brusco et al. ¹⁴
Australia	88	16	6	12	17	2	NP	NP	NR	NP	NP	ND	41	Storey et al. ¹³
China	85	4,7	5,9	48,2	ND	ND	NP	NP	NR	NP	NP	ND	41,2	Tang et al. ¹⁷
Japan (Honshu)	101	ND	5,9	33,7	5,9	NP	NP	NP	NR	NP	NP	19,8	?	Watanabe et al. ¹⁸
Finland	49	4	2	ND	2	12	18	ND	ND	NP	2	ND	61	Juvonen et al. ¹⁹
Germany	77	9	10%	42	22	NP	NP	NP	NR	NP	NP	NP	17	Schöls et al. ²
Norway	48	<1	<1	<1	ND	NP	NP	NP	NR	NP	NP	NP	92	Erichsen et al. ¹¹
India	77	15,6	24,7	2,6	ND	2,6	ND	NP	6,5	NP	NP	ND	48	Srivastava et al. ²⁰

Results are displayed in percent. ND: not detected; NP not performed; und.: undetermined; *of 174 undiagnosed patients, only 87.3% (152 patients) underwent the adopted genetic test, resulting in 26.48% of patients with unidentified mutations.

Table 4. Prevalence of spinocerebellar ataxia in Brazil.

Reference	n	SCA1	SCA2	SCA3	SCA6	SCA7	SCA8	SCA10	SCA12	SCA17	DRPLA	und.
Silveira ²⁸	67	5%	NP	55%	NP	NP	NP	NP	NP	NP	2%	61,20%
Lopes-Cendes ²⁹	54	6%	9%	44%	NP	NP	NP	NP	NP	NP	NP	40%
Jardim ²¹	52	ND	ND	92%	ND	2%	*	NP	NP	NP	ND	6%
Trott ³⁰	114	ND	4,40%	84,20%	1,80%	ND	NP	1,80%	NP	ND	ND	6%
Freund ³¹	115	ND	5,20%	21,70%	0,80%	2,60%	NP	NP	NP	NP	NP	69,50%
Teive ²³	104	2,90%	7,20%	72,50%	ND	4,30%	NP	11,60%	NP	NP	NP	33,70%
Cintra ²²	150	6%	3%	81%	1,50%	7%	0,80%	0,80%	NP	NP	NP	12,70%
Castilhos ²⁵	359	5,20%	7,80%	59,60%	1,40%	5,60%	NP	3,30%	ND	ND	ND	18,10%
Teive ²⁶	460	4,3%	6,5%	45,7%	0,6%	1,8%	NP	18,3%	NP	NP	NP	22,8%
Braga-Neto ²⁷	487	4,30%	11,50%	53,60%	1,20%	4,50%	NP	2,20%	0,20%	ND	0,20%	22,3%

und.: undetermined.

several genes (or DNA templates) simultaneously, providing a large amount of information in an accurate and fast way, whereas Sanger sequencing, despite being reliable, can sequence only one gene at a time, making investigation time-consuming and costly³⁴.

When one suspects of SCA and performs a DNA test such as Whole Exome Sequencing (WES), about 64% of the diagnoses made by this method are from mutations traditionally known to be responsible for causing hereditary ataxias, while 30% are from newly discovered genes and 6% from genes that were not typically considered to cause ataxia³⁵.

In practical terms, NGS can be employed in three ways:

- Targeted sequencing panels (TSP), considered the most cost-effective approach, involving the analysis of a restricted number of genes in coding regions (exons).
- WES, where there is analysis of all coding regions of the human genome, site of about 85% of all pathogenic variants.
- Whole genome sequencing (WGS), considered to be the most expensive method yet capable of detecting mutations in coding (exons) and noncoding (introns) regions, as well as copy number variations (CNV)^{33,36}.

The excess information provided by these methods can also be a trap. Sometimes variants detected in WES may not necessarily be related to the patient's disease, representing incidental and/or non-specific findings. The latter, also known as variants of unknown significance (VOUS), represent variants of a gene found in genetic testing without a known functional or health consequence to the proband. The former represents pathogenic mutations related to other diseases not related to the investigated ataxia, such as the identification of a mutation in the BRCA gene 1 related to breast and ovarian cancer, but not to ataxia. In these cases, it is important to explain to patients and obtain a consent form on the possible risks associated with the incidental findings of genes predisposing to other potentially serious diseases^{37,38} before starting the genetic test. In this context, it is important to highlight the need of gathering clinical data to determine the most likely types of SCA to be investigated in a specific patient⁵.

Other limitations of WES are: failure to effectively identify nucleotide repeat expansions (the major cause of SCA), as well as mutations in GC-rich regions, mitochondrial DNA variants and copy number variations (CNV). They are also subject to sequential reading errors and technical problems such as insufficient depth and coverage³⁴.

Thus, the current recommendations are to search for nucleotide repeat expansions most associated with SCA by the PCR technique initially, taking into account the phenotype and epidemiological contexts. However, after ruling out this as a cause, another 70 genes associated with different forms of ataxias may be involved³⁹. Therefore, if the initial results are negative, alternative methods for diagnosis, such as NGS, should be considered.

THE USE OF NEXT GENERATION SEQUENCING: EVIDENCE OF LITERATURE

Recent studies have shown encouraging results of NGS when confirming diagnosis in patients with hereditary ataxia. Pyle et al.⁴⁰ found pathogenic variants in 41% of patients without diagnosis in 22 families, using the WES method. Efficacy was similar between patients with early onset (<20 years) and late onset (>20 years). Although there was criticism of this study⁴¹, it revealed the potential impact of WES in patients with hereditary ataxias at any age.

In contrast, Németh et al.⁴¹ showed that the TSP method identified 18% of cases in a similar cohort. Larger sequencing of the genome is the likely explanation for the superior results of the study by Pyle et al. Since it allowed the detection of mutations in genes that, although known to cause ataxia, are not considered "ataxia genes" and are therefore not usually included in the gene panels^{39,41}.

Meanwhile, Fogel et al.⁴² used WES and identified a percentage similar to that found by Németh, with 21% of cases identified (16/76) in patients with late-onset cerebellar ataxia, predominantly sporadic.

In a prospective study with patients with progressive cerebellar ataxia, Hadjivassiliou et al.⁴³ investigated 146 patients with TSP and identified mutation in 32% of cases. In another study with 412 patients with a negative molecular diagnosis of ataxia, Coutelier et al.⁴⁴ performed TSP combined with PCR, finding relevant genetic variants in 14.3% of the cases. The same group carried out another study with 319 patients with cerebellar ataxia, with no history compatible with autosomal dominant pattern and undiagnosed, using WES⁴⁵. Relevant genetic variants were identified in 28.5% of the cases (22.6% with definitive diagnosis and 6% with a possible pathogenic variant). In this cohort, younger patients (<25 years) with a history of consanguinity were associated with better chances of diagnosis, which had been previously demonstrated^{46,47,48}. Table 5 summarizes the main mutations found in these studies^{40,41,42,43,44,45}.

Table 5. Main mutations found with next-generation sequencing technology.

Authors	Genes mutations
Coutelier et al. ⁴⁴	CACNA1A (16 cases); Del. ITPR1 (11 cases); SPG 7 (9 cases), AFG3L2 (7 cases)
Hadjivassiliou et al. ⁴³	CACNA1A (11 cases), PRKCG (5 cases), SPTBN2 (4 cases), SPG 7 (4 cases)
Coutelier et al. ⁴⁵	SPG 7 (14 cases); SACS (8 cases); SEXT (7 cases), SYNE 1 (6 cases), CACNA1A (6 cases)
Németh et al. ⁴¹	SEXT (2 cases), TTBK2 (1 case), PRKCG (1 case), MRE11A (1 case), SACS (1 case)
Fogel et al. ⁴²	SYNE 1 (3 cases), SPG 7 (2 cases)
Pyle et al. ⁴⁰	SPG 7 (3 cases), SACS (3 cases), NPC1 (2 cases), TUBB4A (2 cases)

These studies have demonstrated that NGS technologies play a crucial role in the diagnosis confirmation of ADCA, leading not only to a decrease in the time of diagnosis of patients, but also in the correlation of the genotypic-phenotypic spectrum, a source of discovery of new genes that cause ataxia, whether unpublished⁴⁹ or not previously associated with ataxia³⁹. It is important to point out that the studies have heterogeneous populations, and a comparison between them may be statistically inappropriate.

STRENGTHS AND PITFALLS OF WHOLE EXOME SEQUENCING AND TARGET SEQUENCING PANEL

TSP are considered a faster and cheaper method when compared to WES, the former representing a useful tool to identify mutations outside the exons, decreasing VOUS and incidental findings, which are important limitations of WES. In addition, it provides more concise information, which can be complemented with confirmatory methods, such as Sanger's sequencing, which fills any data gaps unread by TSP³³. The great limitation of TSP method is the need to formulate a genetic panel compatible with the phenotype and family history presented by the patient, which depends exclusively on previously reported clinical findings for the selection of genes, which may allow the escape of more rare genes, linked to atypical presentations or new mutations^{35,42}. In addition, new TSP designs are needed as new genes are described.

On the other hand, in WES there is a broad genetic evaluation, without the need for previous clinical information. This allows the discovery of novel genotypic-phenotypic associations, extension of the phenotypic spectrum of a particular gene or recognition of very rare diseases or new mutations³⁹. In addition, it can detect about 100-fold more genes compared to the mean detected by diagnostic panels (100–200 genes). It is an excellent tool for patients with hereditary ataxia, considering the great phenotypic and genotype heterogeneity of these patients⁴³.

Another advantage of WES is the possibility of reanalysis of the previously obtained data as new genes are discovered and disseminated in the scientific community, enabling a retrospective diagnosis³⁴ and reducing time and costs compared to TSP.

Among the problems related to WES we can mention:

- Poorly effective for the diagnosis of nucleotide replications, mutations in GC-rich regions, variants in mitochondrial DNA, structural variations of DNA, mutations in non-coding regions (intronic mutations).
- Incidental and undesired finding of genetic mutations predisposing to cancer, Alzheimer's or other degenerative diseases.
- Generation of large number of variants, which requires the sequencing of family members to "filter" variants of

uncertain meaning, reducing specificity and increasing the cost of the procedure.

- To establish a genotypic-phenotypic relationship of a new or non-associated variant prior to ataxia through bioinformatics processing, which may be highly complex.
- Technical problems, such as reading errors, coverage and insufficient depth — the most commonly problem associated with loss of variant detection — may compromise results^{34,40,41,50}.

WGS is a method that was restricted to research centers, however, it has been more and more used in the routine of genetic laboratories worldwide. It is known that WGS has a much broader coverage of coding regions compared to WES, as well as covering non-coding regions. However, the amount of information generated may require a lot of time for analysis, considered highly complex, and the cost is much higher than WES. Also, WGS have the same limitation of WES and TSP in detecting repeat expansions⁵⁰. Figures 1, 2 and 3 summarize the main advantages and disadvantages of the NGS methods^{34,35,36,39,42,45,47,50,51}.

It is important to note that although some studies points WGS to be a cost-effective approach⁴⁶, it is still an expensive and unavailable method for most patients in Brazil.

SHORT TANDEM REPEAT EXPANSIONS AND NGS: SOLUTIONS

As mentioned throughout the text, NGS methods are not suitable for STR identification. This is due to the fact that currently available methods perform short readings (about 150bp per reading) and the STR expansions responsible for SCA, with few exceptions, usually have expansions that go beyond this limit.

In order to solve this problem, in recent years analysis methods have been developed, such as ExpansionHunter⁵², exSTRa⁵³, STRetch⁵⁴ and TREDPARSE⁵⁵, which applied together with NGS, are capable of detecting STR expansions where the expanded allele size is greater than the length of standard short-read sequencing reads.

In the past, there were other detection methods for STR, such as HipSTR and LobSTR, but both have the limitation of detecting only STR alleles with repeat lengths smaller than the read length employed in the sequencing. All methods except exSTRa perform better when are applied to a WGS platform, preferably PCR free, where library preparation protocols yield the best data to allow repeat expansion detection, although platforms such as WES provide enough data to detect expansions in STR loci.

Dashnow et al demonstrated the use of STRetch in four patients without diagnosis after screening for most common expansions (SCA1-3, SCA 6, SCA 12, SCA 17 and DRPLA) and use of WGS screening for SNV. STRetch was capable of

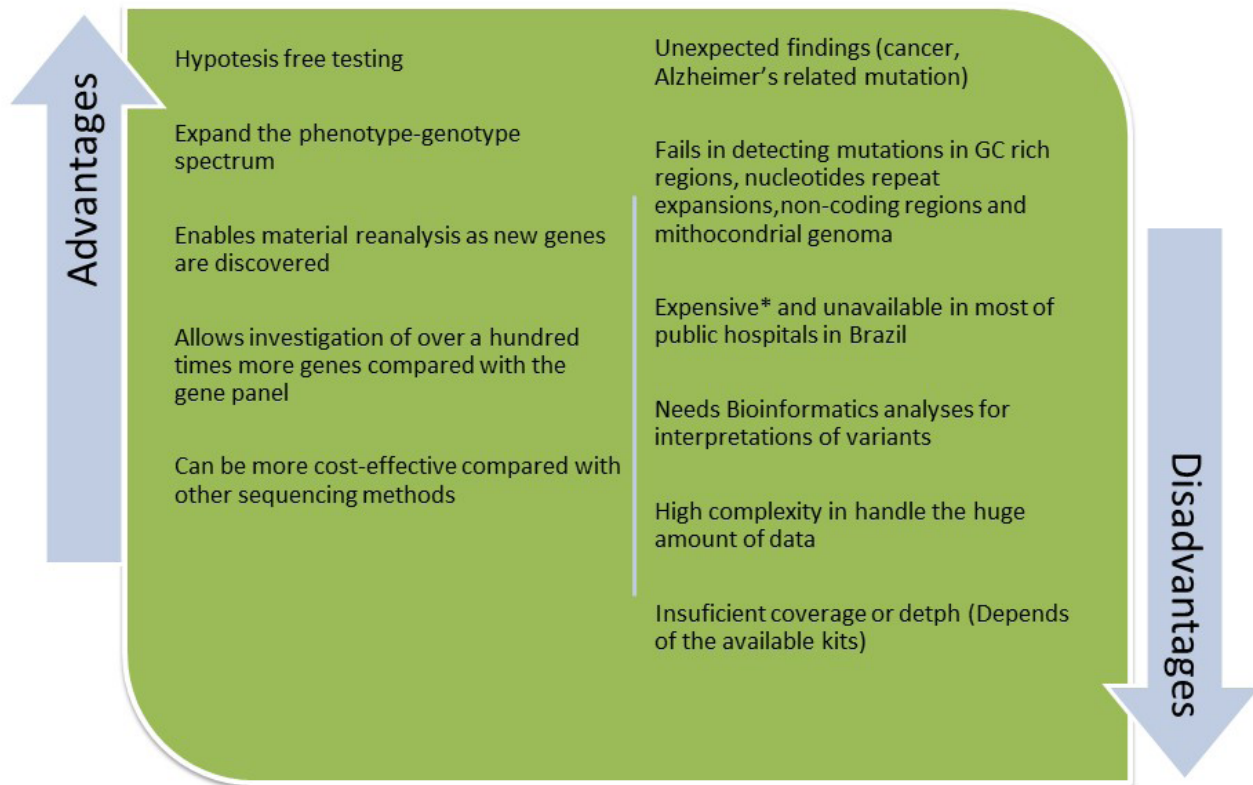


Figure 1. Whole exome sequencing^{34,35,36,39,42,45,47,50,51}.



Figure 2. Target sequencing panel^{34,35,36,39,42,45,47,50,51}.

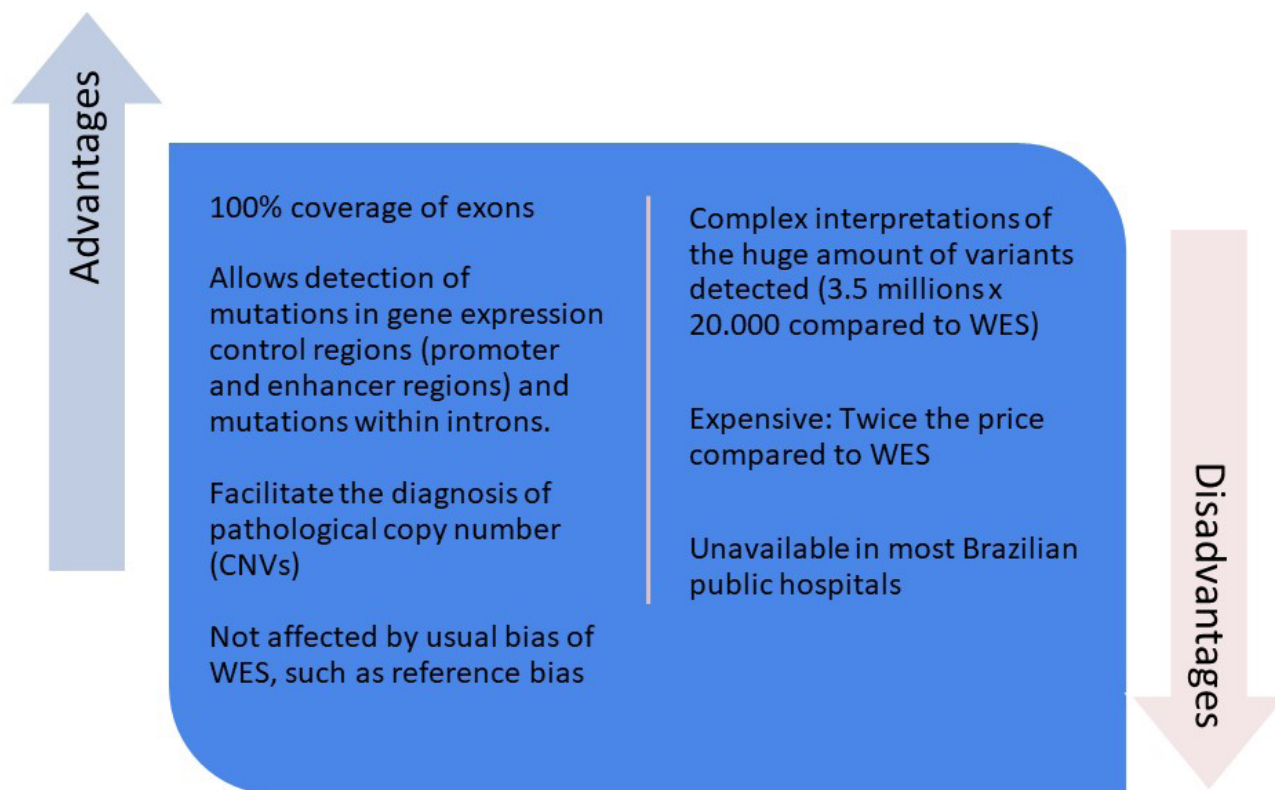


Figure 3. Whole genome sequencing^{34,35,36,39,42,45,47,50,51}.

identifying a SCA 8 expansion in one patient, confirmed by PCR⁵⁶. Tankard et al. compared all four methods in different NGS platforms for detection of expansions of tandem repeat, and showed good sensitivity and specificity for all of them (>87 and >97%, respectively, when the methods were applied with WGS PCR free platform) and none of them were better than the other, suggesting that the use of all existing methods could be advantageous, improving the accuracy of the results⁵⁷. Each of these alternative methods has its own technical advantages and disadvantages that goes beyond the scope of this review and must be seen elsewhere.

Although these techniques can detect novel repeat expansions, all of them rely on a priori knowledge of STR loci to be examined, that can be assembled by using annotation of STRs from Tandem Repeats Finder results. Hence, de novo mutation cannot be detected by these techniques yet. Furthermore, some STR loci are poorly captured due to their extreme GC content, such as repeat expansions alleles underlying FRAXA (FMR1), FRAXE (FRM2) and FTDALS1 (C9orf72). Despite these limitations, several authors recommend their implementation in routine screening with NGS⁵⁸.

It's important to remember that gold-standard techniques for diagnosis of STR expansion, such as Southern blots and TP-PCR (Tripled Primed PCR) shouldn't be abandoned. The new NGS technique are considered screening methods, requiring validation with gold-standard methods.

Southern Blot or TP-PCR are still the most accurate methods for detecting STR expansions and the size of the expanded allele, including whether there are interruptions, which has prognostic implications for age of onset, disease progression, and outcome^{57,58}.

LONG READ SEQUENCING: A FUTURE NOT SO DISTANT

Sometimes even after extensive investigation with NGS short-read technologies the diagnose remains unknown. This is particularly true in cases with complex expanded alleles^{58,59}, where the repeat may be interrupted multiple times. In this case, long read sequencing could be useful. These technologies, such as PacBio and Nanopore sequencing are gaining notoriety and drawing interest in bioinformatics. Readings can reach tens of thousands in comparison with few hundreds in short-readings NGS. Rather than estimating an STR expansion, the LRS will capture the entire expanded allele in a read fragment, providing more accurate information about that expansion. While encouraging, LRS is still considered very expensive (about 10x more compared to conventional NGS methods) and is therefore not cost effective for routine use^{57,58}. However, this should change soon when LRS will be a valuable tool for the diagnosis of Mendelian diseases such as ADCA.

CONCLUSION

Hereditary ataxias are a complex group of diseases from a clinical and genetic point of view. About 30% of patients with ADHA remain undiagnosed after an initial investigation into the most common gene variants. Guidelines for the investigation of SCA recommend that the initial investigation be done according to the phenotypic characteristics and family history, which may favor one type of SCA compared to others^{60,61}.

Although there are limitations, studies have shown that the use of NGS may be useful in the investigation of patients with undiagnosed ataxias. The most common mutations related to SCA are due to the expansion of nucleotides, which is a limiting factor in NGS technologies. However, in the context of a negative molecular diagnosis of ataxias, several other

molecular variants such as deletions, missense, nonsense and splice mutations (SCA 5, 11, 13, 14, 15/16 and 27), mutations in non-coding regions (SCA 8,10 and 12) or mutations associated with other diseases such as spastic paraplegias, recessive ataxias, and channelopathies, may be responsible. In these cases, and NGS have proven effective in accelerating the diagnostic process.

Furthermore, new techniques for detections of STR expansions with NGS, such as exSTRa, STRetch, ExpansionHunter and TREDPARSE, are proving to be valuable tools in diagnosing STR related diseases, which includes SCA^{56,57,58}. Long read sequencing it's another promising diagnostic method for mendelian diseases, but it's not widely available and it's too expensive for routine use in clinical practice.

The unbridled evolution of neurogenetic research may answer many current questions soon enough.

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