Review

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Current molecular insight to reveal the dynamics of CAG repeating units in spinocerebellar ataxia

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Summary **Spinocerebellar ataxia (SCA) is a heterogeneous genetic disorder with overlapping clinical phenotypes arising from the degeneration of purkinje cells and other regions of the brain. There are approximately 36 different subtypes of SCA, but SCA 1, 2, 3, 6 and 7 are most prevalent in the Indian population. Many findings suggested that cerebellar Purkinje cells region may be a uniquely vulnerable neuronal cell type, and more susceptible to a wider variety of genetic or cellular problems than other neuron types. In this review we emphasized mainly five common subtypes of SCA (1, 2, 3, 6 and 7) their pathophysiology, therapeutics, drugs studies and the technical challenges in the field of molecular genetic diagnosis.**

Keywords: Spinocerebellar ataxia, SCA, Triple Primed PCR (TP-PCR), polyglutamine disease, Autosomal Dominant Cerebellar Ataxia (ADCA)

1. Introduction

Spinocerebeller ataxia (SCA) is a rare, autosomal dominant, neurodegenerative disorder. SCA is mainly caused by a triplet repeat expansion and sometimes repeats can be pentanucleotide and hexanucleotide, however SCA can also be caused by point or missense mutations (*1*). Until now 40 different subtypes of SCA have been reported. Though the disease is mostly adult onset a few subtypes of childhood onset are also seen (*2*). SCA including subtypes 1, 2, 3, 6 and 7 are cause by the triplet Cytosine Adenine Guanine (CAG) repeat expansion in the precise genes that leads to the formation of an abnormally long polyglutamine chain in the respective encoded proteins (*3*).

SCA affects brainstem, spinal cord, cranial nerve nuclei, and cerebellum that finally lead to the progressive cerebellar ataxia, gait disturbance, nystagmus, dysarthria, tremor and ophthalmoparesis. Cerebellar degeneration does not only lead the movement problems but it also increases the variability and unbalancing of the whole body movement (*4-6*).

Clinical diagnosis of SCA subtypes is challenging because of the coinciding clinical features in different subtypes. To overcome these issues ADCAs (Autosomal Dominant Cerebellar Ataxia) classification of SCAs includes three major categories, Based on inheritance pattern and clinical features (*7*). Later on, the classification of ADCAs was little modified by Duenas *et al*. (*8*), on the basis of neuropathological features that provide a suitable characterization in clinical practice to facilitate genetic diagnosis. These categories are ADCA I, ADCA II and ADCA III. In ADCA I category SCA subtypes in which neuro-degeneration takes place just outside the cerebellum region, similarly for ADCA-II and ADCA-III neurological features with retinal degeneration and degeneration is only restricted to the cerebellum region respectively because degeneration is only restricted to cerebellum region for ADCA III category called pure form of the ataxia. The paternal transmission is mostly associated with the occurrence of repeat expansions in the next generation rather than maternal transmission of the expanded allele (*9*). The duration of disease is 10-15 years after onset. Now in this review we will discuss the five common subtypes of SCAs.

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2. Subtypes (SCA 1, 2, 3, 6 and 7) and their pathophysiology

2.1. *SCA1*

First case of SCA 1 was reported in the year 1993. SCA 1 appears in mid-30s and is characterized by gait disturbance, dysarthria and ocular Dysmetria which progresses to ophthalmoplegia (*10*). It is caused by CAG repeat expansion in coding region of *ATXN1* gene that encodes Ataxin-1 protein. The trinucleotide CAG repeat size for normal (unaffected), intermediate and affected alleles are ≤ 36, 36-44 and ≥ 44 respectively. Cytosine Adenine Thymine (CAT) interruptions (1 to 3 in no.) may be present in normal individuals, but in affected individuals the expanded repeats are present continually (*4*).These interruptions provide repeat size stability by avoiding the expansion of CAG repeats during (Deoxyribonucleic Acid) DNA replication (*11,12*). The mutated Ataxin-1 protein has an unusually long stretch of polyglutamine (CAG repeat encoded) that changes its 3D structure leading to abnormal interaction and aggregate formation with other nuclear proteins.

These aggregates decline to allow accurate functioning, which finally damages the purkinje cells, and finally leads to cell demise or degeneration of brain cells. Cytoplasmic localization unlike normal protein remains in the nucleus, so the problem is in cerebellar Purkinje cells that leads to progressive degeneration of the Purkinje cells.

The expanded *ATXN1* gene's alleles are similarly translated into proteins of apparently normal stability and distribution. The aggregates of protein are found only in the brain and spinal cord (CNS) region of the brain (*3,13*). Therefore, the cells within the cerebellum are very sensitive particularly to the alteration in ataxin-1 protein shape, and loss of cells of the cerebellum (Table 1).

2.2. *SCA2*

SCA2 first case was reported by Wadia and Swami and therefore this subtype also is called the Wadia-Swami Syndrome. It is the most prevelent form of SCA in the Indian population and is characterized by progressive cerebellar ataxia, Nystagmus and slow saccadic eye movements appears mostly in the 4th decade of life. It is caused by CAG repeat expansion in coding region of the *ATXN2* gene that encodes Ataxin-2 protein which is involved in cytoplasmic (Ribonucleic Acid) RNA-related functioning (*14-16*). The CAG repeat size range for unaffected individuals is ≤ 31 with (Cytosine Adenine Adenine) CAA interruptions while affected individuals, have an expanded uninterrupted region of more than 33 CAG repeats (*17*). The presence of CAA interruption does not ease the severity of pathogenicity as it also encodes for glutamine like CAG (*18*); yet

Table 1. Genetics of SCAs subtypes

Subtype	Chromosome Location	Gene	Exon (Mutation present)	Age onset	Distinguishing features (Including Ataxia)	Triplet Repeat	Repeat Size (Normal)	Repeat Size (Expanded)	Protein	Protein Location	Duration of disease (Years)	Affected Brain region
1	6	*ATXN1*	8	3rd – 4th decade	Active Reflexes	CAG	6 - 36	44 - 83	Ataxin 1	Nucleus & cytoplasm	15	Cerebellum
2	12	*ATXN2*	1	3rd – 4th decade	Slow saccadic eye movement	CAG	15 - 31	34 - 220	Ataxin 2	cytoplasm	10	Cerebellar purkinje cells
3	14	*ATXN3*	10	3rd – 4th decade	Muscle weakness and atrophy	CAG	12 - 40	54 - 86	Ataxin 3	Nucleus & cytoplasm	10	Ventral pons and Substantia nigra
6	19	*CACNA1A*	49	5th – 6th decade	Very slow progression of disease	CAG	4-18	21 - 33	Alpha-1A calcium channel protein	Membrane associated	> 25	Cerebellar purkinje cells
7	3	*ATXN7*	5	3rd – 4th decade	Visual loss	CAG	4-19	33 - > 300	Ataxin 7	Nucleus and cytoplasm	20	Cerebellar purkinje cells, Brain stem, Spinal cord

these interruptions may enhance the meiotic stability of the repeats (*19*). Previous studies have depicted that the absence of CAA interruption in expanded alleles may increase its instability leading to elevated risk of transmission of a larger expansion in the next generation. The affected Ataxin-2 protein finally targets the different pontine region and Purkinje cells in the cerebellum, and this protein is confined to the RNA containing stress granules, which is related to the endoplasmic reticulum/Golgi segment and plays a very significant role in cytoplasmic RNA-related functions (*14-16*) (Table 1).

2.3. *SCA3*

It is the most common subtype with clinical features, such as progressive ophthalmoplegia, ataxia, basal ganglia symptoms, pyramidal signs, dystonia, dysarthia and distal amyotrophies (*20,21*). It was first discovered in the family of Machado and Joseph and therefore this subtype is also called Machedo Joseph Disease or *MJD*. Normal repeat size for this subtype is ≤ 44 intermediate range and the mutated repeat range is 44 to 52 and 60-84 CAG repeats respectively. The SCA 3 causing gene is *ATXN3* that encodes the Ataxin3 protein. Over repetition of CAG in the *ATXN3/MJD1* gene ultimately translates into an affected Ataxin-3 protein causing neurotoxicity that might develop because of proteolysis of the main protein to liberate the expanded repeat fragment. The aggregation of proteins that is the hallmark feature of this subtype and also in this subtype Calpains (calcium-dependent cysteine proteases) initiates toxic fragment formation and ultimately leads to neuronal loss or degeneration (Table 1).

2.4. *SCA6*

It is also a common subtype of SCA and is characterized by cerebellar ataxia, dysarthria, and Nystagmus, with very slow progression that is the hallmark feature of this subtype (*22*). The CAG repeat ≤ 18 reported as the normal repeat size and 19 repeats is considered as the intermediate repeat size. In this subtype the repeat expansion mutation in the *CACNA1A* gene that encodes the $Ca_v2.1$ calcium channel protein. This gene has two splice site forms, Q-type and P-type isoforms and the CAG repeat expansion falls mainly in the P-type. This type also affects cerebellum where Purkinje cells are found within the Purkinje cell layer. In SCA6 affected individuals the Purkinje cells in, mutant $Ca_v2.1$ proteins form ovular intracellular inclusions. In study of cell culture models of this disease it presented early apoptotic cell death. Voltage-dependent calcium channels are the hetero-oligomeric proteins that comprise pore-forming α1 and auxiliary β, α2 and δ and in some tissues γ subunits (*23,24*).

As these calcium channels facilitate the entry of calcium into the cells in response to change in membrane potential so the disturbances in the Voltage-dependent calcium channels cause a number of neurological difficulties, such as epilepsy, migraine and cerebellar ataxias (*23,24*). An expanded CAG repeat in the *CACNA1A* gene results in a lengthened polyglutamine tract in the C-terminal region of the α1A subunit of *CACNA1A* protein. The heterologous expression of mutated α1A subunits enhances calcium channel deregulation, and finally interferes with the calcium homeostasis in Purkinje cells (Table 1).

2.5. *SCA7*

It is the fifth most common subtype of SCA with pigmented retinal atrophy as a distinguishing feature. The progression of this subtype is often more rapid and aggressive in children than adults. The clinical diagnosis is a little difficult in newborns because the ataxia and visual loss are not a very obvious symptom in SCA7 and failure to thrive and loss of motor milestones may be the initial symptoms (*25*). SCA7 causing gene *ATXN7*, is a polymorphic CAG repeat tract that falls in the first exon, while the normal allele size is between 4 to 19 and abnormal allele size is ≥ 37. The encoded protein is a component of SPT3/TAF9/GCN5 acetyl transferase (STAGA) and TBP-free TAF-containing (TFTC) chromatin remodeling complexes, and it plays an important role in the transcription regulation process. The process of Protein formation in affected individuals detected in the nuclear fraction appears to be ~130KD in size (*26*). The CAG repeat expansions in *ATXN7* decreases the transcription of an antisense non-coding RNA that promotes the repressive chromatin modification of the ataxin-7 promoter region (*27*) that leads to an increase in expression. Normal allele size is ≤ 36 and pathogenic allele size is ≥ 450 CAG repeats. Expansion mutation in *ATXN7* gene suppresses the transcription of an antisense non-coding RNA that promotes the repressive chromatin modification of the ataxin-7 promoter that leads to over expression of mutated protein (Table 1).

3. Genetics

It is a rare genetic disorder and the inheritance pattern is autosomal dominant. The CAG repeat expansion in the particular gene that present at specific locations of the chromosome and that gene codes for a particular amino acid, glutamine. Expansions cause the formation of glutamine expanse or polyglutamine tract. In this disease the gene involved in the formation of ataxin-1, ataxin-2, ataxin-3, ataxin-7 for SCAs 1, 2, 3, 7 respectively and the location of these genes is 6p, 12q, 14q, 3p respectively and for the SCA 6 subtype gene is *CACNA1A* and location of this gene is 19p (Table 1).

The proteins encoded by these genes is involved in

destroying and getting rid of the surplus, damaged or unneeded proteins that presents in the cells. The role of Ataxin proteins is to eliminate the ubiquitin from these unwanted proteins just before they are ready for degradation so that the ubiquitin can be used again. Ataxin proteins are also involved in regulation of the first stage of protein formation (transcription). In the case of SCA6 because of the expansion mutations in the *CACNA1A* gene, encodes for the protein that acts as a pore forming α1A subunit of P/Q type calcium channels and is responsible for starting and regulation of synaptic transmission (*28*).

4. Diagnosis

Diagnosis of Spinocerebellar ataxia is primarily based on the clinical characteristics and the next need is for evidence of family history. Like many diseases with known genetic causes, a family history that can disclose multiple family members with similar clinical conditions can easily indicate the diagnosis of SCA. However, an ataxic patient whose family history constitutes a genetically confirmed diagnosis of a spinocerebellar ataxia subtype is a perfect candidate for genetic testing, but such types of cases are not very constant. If the movement related problems existed previously in the family record, a previous diagnosis is likely to show a classification given to the disease at the time of diagnosis. So the diagnosis of SCA divided into the two types first is clinical diagnosis and second is molecular genetic diagnosis.

4.1. *Clinical diagnosis*

For clinical diagnosis if the patient presents ataxic features like movement problems, Nystagmus, dysphagia, dysarthia *etc*. then there is a requirement to check this through the help of some neurological testing like CT scanning and Brain MRI (*29*), because in SCAs degeneration of cerebellum is present. So if shrinkage of cerebellum is manifested in CT scanning and Brain MRI then next molecular confirmation and subtyping of SCA needs to continue for molecular genetic diagnosis.

4.2. *Molecular diagnosis*

The molecular diagnosis of SCAs relies on the tests that determine the number of the triplet (CAG) repeat elements in the particular gene. Southern blot analysis was the first method but It has some drawbacks because this method is expensive, time consuming, radioactive based and requires a large amount of DNA concentration for a single reaction. Polymerase Chain Reaction (PCR) was used to detect the triplet repeat expansions that are less than ~100 repeat (*30*), the PCR technique can reveal the pattern of alleles either two heterozygous peaks of normal-sized alleles or a single homozygous peak normal-sized. When the repeat expansions number more than 100 repeats, or fall outside of the detectable range by PCR and would require Southern Blot analysis or triplet primed PCR, for routine testing of expansion mutations you can usually perform either Southern blot analysis (*31,32*) or a long-range PCR method (*33,34*). However long-range PCR is cost effective and much faster than southern blotting.

Short PCR method for routine diagnosis of the larger triplet repeat mutations in Indian Triplet repeat disorder patients is recommended as a best method for molecular diagnosis for Triplet repeat disorders (TRDs) in a very fast and cost effective manner. By Normal or Short PCR analysis we cannot detect the repeat expansion of ≥ 100 repeats so in such a case a useful method is TP-PCR (triple primed PCR), to detect triple repeat expansion in cases of more than 100 repeats. TP-PCR (Triplet repeat primed PCR) is a new and advanced technique to detect large triplet (CAG) repeat expansion mutations in (Mytonic Dystrophy) DM1 and SCA patients (*35*). In the Indian scenario previously many studies have been described roughly about the usefulness and benefits of the TP-PCR method for quick diagnosis and proper genetic counseling in triplet repeat disorders (*36*). TP-PCR is very beneficial explicitly for the uncovering of repeated expansion mutations.

5. Treatment and management

Inopportunely, there is no proper treatment available for SCAs but for management help using cane sticks and walkers may be helpful to prevent falls of ataxic patients, and prepare ramps for mechanized chairs. Generally, treatments are directed towards progression of the symptoms, not for the disease itself. Some therapies and medications might be appropriate for some of these particular symptoms, like depression, spasticity, tremors, sleeping problems and some others (Table 2).

Voxel-based morphometrics can also expose the volume loss in cerebellum and brain stem region involving both gray and white matter of the brain (*37,38*). The region degeneration may also be able to be seen by this method (*39*). Some other landmarks such as the measurements of some metabolites such as myo-inositol and -acetyl aspartate that can disclose the evidence of neuronal cell loss in the pons and cerebellum and even the supratentorial structures of brain (*40*). Loss of cerebellar and brain stem grey matter and motor dysfunction problems disclosed by quantitative imaging studies have been recently documented in pre-symptomatic persons known to have an *ATXN1* triplet repeat expansion mutation.

A number of therapies and educating places should be available for ataxic and dysarthria patients. These therapies should include, phonological therapy, writing

Table 2. Main symptomatic treatment proposed for patients with autosomal dominant hereditary ataxias

Symptomatic treatment (Drugs)	Ataxia type
Riluzole 100 mg/day	SCAs and other etiologies (recessive and sporadic)
protirelin tartrate or taltirelin hydrate	SCA1
protirelin tartrate or taltirelin hydrate	SCA2
Varenicline 1 mg twice day	SCA3
Buspirone 30 mg twice daily	SCAs
Oral zync 50 mg/day	SCA3
Insulin-like growth factor-1 A	SCA3
Mexiletine and Carbamazepine	SCA3 (pain and cramps)
Botulinum toxin type A	SCA3 (dystonia and spasticity
protirelin tartrate or taltirelin hydrate, Acetazolamide, gabapentin and pregabalin	SCA6
protirelin tartrate or taltirelin hydrate	SCA7

therapy, speech therapy and rehabilitation, different work-related therapies, dietetics essential to give them psychosomatic care and also support from social services. Towards the direction of treatment with the help of therapies should be communication devices, should train patients to carry their eating utensils and also have dressing hooks for help to make them self-dependent and can explain coordinative physiotherapy. Many SCA patients have other symptoms, in addition with the ataxia so some medications and some other therapies might be helpful for these symptoms. The noxious protein oligomers potentially can be reduced through proper stimulation of Heat Shock Protein (Hsp) members, although reducing the level of transcriptional deregulation and RNA aggregation may also be helpful in progression of disease. Compounds which can limit the intensity of the agitation of Purkinje neuron cells and reduce the level of release of intracellular calcium, have been established as advantageous in multiple model studies of different subtypes of SCAs. Compounds such as SK channel activating compounds and dantrolene, might recover the function of purkinje cells by adaptable pacemaker firing disturbances and could diminish the stimulation or activation of Ca^{2+} dependent cell death processes that ultimately leads to neuronal cell deterioration. Numerous clinical trial studies revealed the success of Riluzole drug and showed SK channel stimulation may have a particular beneficial effect for the purpose of treatment of several etiologies of SCAs.

Some neuro-protective medications or drugs (*N*-methyl-D-aspartate antagonists) are even now available in phase trials. So development of a reliable ataxia rating scale to screen disease progress and treatment responses has been started. Forthcoming convenience of the therapeutic interventions would slightly change indications of the DNA testing and its psychological and social impacts.

6. Genetic counseling and Preimplantation genetic diagnosis

As previously described its inheritance pattern is autosomal dominant means that if any patient's parents have a mutant allele, there is 50% chance to the sibs to inherit the mutant alleles. For this reason, many couples with affected parents mostly choose to not plan for a child in the future. For the last two decades developmental of Preimplantation genetic diagnosis (PGD) which contains testing of the fertilized ova (in vitro fertilization (IVF)) to distinguish mutation in the affected gene, and after implantation of selected particular healthy embryos to ensure that the presence of pathogenic alteration from parents will not be transmitted to the next generation (*41*). The genetic test must be achieved in respect to formal genetic counseling. This testing is not very beneficial in estimating the severity, age of onset, symptoms types or rate of progression in individuals who are asymptomatic.

7. Prevalence

The prevalence of SCA with great accuracy is a very difficult task to clarify because in most of the studies it is explained various ways. First subtype of SCA is reported from diverse ethnic groups worldwide with varying prevalence, SCA1 estimated prevalence is 22% of total ADCAs (Autosomal Dominant Cerebellar Ataxia) in India. So the prevalence of some late onset SCAs may be underestimated, however, on the basis of available reported studies, SCA different subtypes such as 1, 2 and 3 accounts for the most prevalent in the whole world's population. SCA 3 subtype first originated from two families of the MJDs, who were the Azorean descents found in different ethnic populations and were found to be the most common in different countries like Germany and US. SCA2 subtype is the most common subtype in countries like southern Italy, Spain, Cuba and India. Dentatorubral - pallidoluysian atrophy (DRPLA) is most reported from Japan and is very rare in North America, so it is most prevalent in Japan (*42-51*). Another scientist Soong and his colleagues reported SCA3 was the most common subtype of Autosomal Dominant cerebellar ataxia (ADCA) in Taiwanians (47.3%), next followed by subtype SCA6 (10.8%), SCA2 (10.8%), SCA1 (5.4%), SCA7 (2.7%), SCA8 (2.7%), and DRPLA (1.4%) and

rare in Indians.

In another study from Singapore, scientists Zhao *et al*, reported that prevalence of this disease among Singaporean populations was about 1 out of 27,000 population (*52*). Researchers observed a founder effect for the specific subtypes of SCA, On the basis of history and ancestry of spinocerebellar ataxia (SCA), as well as the correlation between subtypes and ethnicity. So the global prevalence of SCAs fluctuates from 0.3 to 3.0 per 100,000 population (*53*).

8. Conclusion

Numerous SCA causing mutations are currently known, nonetheless there are many other mutations that remain unknown. So the target for convergent mechanisms of neuronal dysfunction in Ataxia needs to be a most effective therapeutic mediation in the near future. It has been more than one decade since the discovery of SCA but the disease continues to hold surprises in spite of extensive research in this field. Among the primary goal of the researchers, it is to find effective targeted therapy for this disease and also to develop speedy, sensitive and cost effective genetic diagnostic methods. In present review, all the insight of genetics, treatment, medications, therapies and diagnosis of the five most common subtypes of SCA is discussed. More research is required to reveal the precise drugs and proper treatment for designing and validating possible drug targets. Succeeding advancement in therapy, early recognition of the disease is also a big concern. The early molecular diagnosis of SCA is too important since to ensure not only affected persons and also their members who can receive all possible benefits through genetic diagnosis, including genetic counseling that is very important for prenatal diagnosis for the risk of recurrence of SCAs high in the family and their relatives.

TP PCR has preferably substituted traditionally used techniques owing to its sub sensitivity, selectivity, and very low cost. It offers the possibility of early diagnosis in clinical suspects, and prenatal testing. Progress in both genetic diagnosis and therapy would hopefully improve the quality of life for the SCA patients in the near future.

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