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FGF14 Non-coding Repeat Expansions and Ataxia

Current Understanding and Research Gaps

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Abstract

Ataxia is a progressive neurological disorder affecting voluntary muscle movements, often resulting from cerebellar damage. Causes can either be genetic or acquired. The reasons range from genetic mutations to environmental factors. SCA27 is a rare type of spinocerebellar ataxia associated with intronic GAA repeat expansions in the *FGF14* gene. SCA27 shows clinical features of muscle stiffness, gait instability, and tremors that mostly start during adulthood. The GAA expansions interfere with the expression of FGF14 protein, resulting in neurological symptoms, such as impaired neuronal excitability and plasticity. Recent publications have identified *FGF14* mutations in many cases previously diagnosed as idiopathic ataxias, underlining its diagnostic relevance. Repeat expansions of non-coding DNA can lead to gene dysregulation through mechanisms such as transcriptional silencing, RNA toxicity, or cryptic splice site generation. Although some progress has been made in determining the role of *FGF14* in ataxia, the exact mechanisms of neuronal degeneration remain elusive. Future research should focus on mechanisms of neuronal degeneration, gene and RNA-targeted therapy strategies, and understanding the large genetic landscape. These gaps in knowledge would thus have to be closed to help formulate an effective diagnostic and therapeutic strategy for SCA27 and related ataxias.

Introduction

Ataxia

Ataxia is a progressive neurological disorder affecting a person's voluntary muscle movement ability. People with ataxia can lose coordination and balance making it hard to walk. Additionally, it can affect any part of the body leading to fine motor skill deterioration and speech impediments (De Silva et al., 2019).

Usually, ataxia arises as a consequence of damage to the cerebellum, a part of the brain that is responsible for motor control. Many factors can cause ataxia, such as genetic mutations, stroke, alcohol misuse, exposure to toxic chemicals, vitamin deficiencies, as well as other underlying neurodegenerative diseases (Farghaly et al., 2011). Ataxia can arise as a symptom of other conditions or as a disorder on its own. Depending on its etiology, it can be either a progressive, incurable disorder, or its effects can be treated and reversed.

Sporadic and acquired ataxias can develop as a result of genetic factors such as de-novo mutations, or non-genetic factors - traumatic brain injuries, stroke, brain tumors, vitamin deficiencies, or infections (Lieto et al., 2019). These types of ataxias can occasionally be treated, depending on the cause. Inherited ataxias, such as autosomal recessive cerebellar ataxias (ARCAs), spinocerebellar ataxias (SCAs), and mitochondrial ataxias are caused by inheriting mutated genes from one's parent in an autosomal dominant or recessive pattern (Jayadev & Bird, 2013). Nucleotide repeat expansions are the most common cause of the hereditary type.

Non-coding repeat expansions

Repeat expansions can be coding or non-coding. Coding repeat expansions occur within genes and lead to excessive repeat sequences of coding DNA. Non-coding repeat expansions, on the other hand, happen outside genes, in the non-coding part of the DNA sequence. These mutations can disrupt gene regulation and expression in many ways.

Transcriptional silencing happens when the repeat expansion creates a condensed chromatin structure, leading to reduced gene activity. This is the case in the example of Friedrich's Ataxia (FA), where GAA repeats lead to abnormal epigenetic changes and a reduced level of frataxin protein (Sandi et al., 2013). Another mechanism is RNA toxicity. The expanded RNA molecules can form abnormal secondary structures which aggregate into foci and attach to RNA-binding

proteins (RBPs), leading to disruptions of RNA processing and cellular dysfunction (Swinnen et al., 2020).

Additionally, repeat expansions can create sequences that resemble splice sites called cryptic splice sites. Ultimately, the mis-spliced RNA can be translated into a non-functional or even toxic protein (Reis et al., 2022).

Furthermore, repeat length and locus also greatly influence the progression of the disease, leading to either mild or severe phenotypes. Trinucleotide repeat expansions are the most common, but longer repeats, such as tetranucleotide, hexanucleotide, and dodecamer repeats are also associated with disease (Srinivasan et al., 2023). Non-coding repeat expansions located in the 5'UTR regions of the gene are generally associated with increased methylation and gene silencing, while 3'UTR expansions are associated with RNA toxicity (Depienne & Mandel, 2021).

FGF14

Spinocerebellar ataxia type 27 (SCA27) is caused by the intronic GAA repeat expansions in the gene *FGF14* (Van De Warrenburg & Kamsteeg, 2024). This gene is responsible for many biological processes, such as neuronal signaling pathways and synaptic plasticity. The repeat expansion is associated with a decrease in neuronal excitability and synaptic transmission, as well as impaired neuronal signaling pathways resulting in progressive SCA27 (Groth & Berman, 2018).

SCA27 and its association with *FGF14* were only recently discovered, offering a diagnosis to over 30% of patients with previously assumed idiopathic ataxia (Van De Warrenburg & Kamsteeg, 2024).

SA27 is a rare type of adult-onset ataxia that can present sporadically or in episodes and is characterized mainly by muscle stiffness, difficulty walking and speaking, vision problems, tremors, and dizziness. Its late onset and episodic symptoms made this type of ataxia particularly hard to diagnose before its association with the *FGF14* repeat expansion. Studying *FGF14* repeat expansion in more detail is crucial for understanding the disease mechanism and finding potential therapeutic strategies.

Research Question and Objectives

The causes of SCA27 and other ataxias are diverse and not well understood, and the treatment options are very limited. This is why this study aims to elucidate the mechanisms by which the

non-coding repeat expansion of *FGF14* causes ataxia and what are the current gaps in knowledge.

A thorough review of the literature will be conducted, to establish a clear picture of the current state of research and identify areas where our knowledge is limited. Next, the existing theories and models from different studies will be analyzed to assess which mechanisms are the most plausible in the pathogenesis of this disorder. The unknowns in the existing models will be highlighted to bring out the potential gaps in knowledge.

Finally, based on the findings of the literature review and analysis, new research directions will be proposed, which could fill in the gaps identified previously.

By better understanding this debilitating disorder, new diagnostic tools and therapies can be developed which can greatly improve the diagnostic, management, and treatment options available for patients.

Research Findings

Ataxia

Classification and Types of Ataxias

Ataxia can be classified according to the underlying cause and mode of inheritance. Broadly, it is divided into three forms, that are acquired, genetic/hereditary, and idiopathic (Gorcenco et al., 2024).

Acquired ataxia is caused by exogenous factors such as vitamin deficiencies (e.g. B12), infections, autoimmune diseases, toxins, or head injuries (Lin & Kuo, 2023). It is not hereditary and most of the time appears as a result of exposure to an etiological agent.

Genetic ataxias arise due to mutations that were inherited and further can be grouped into autosomal dominant, autosomal recessive, X-linked, and mitochondrial ataxias (Hersheson et al., 2012). Autosomal dominant ataxia, also known as Spinocerebellar Ataxia (SCA), is caused by mutations of several genes, which lead to various subtypes of SCA. Among these are SCA1, SCA2, SCA3, and more. Often, the mutations are CAG trinucleotide repeat expansions that lead to polyglutamine stretches in the proteins encoded (Lieberman et al., 2019). These proteins are toxic to neurons.

Friedreich's ataxia is the most common type of autosomal recessive ataxia. It involves mutations in the *FXN* gene that give rise to lower levels of frataxin, a mitochondrial protein. Its deficiency

impairs the mitochondria and results in oxidative stress and the death of neurons (Cook & Giunti, 2017).

X-linked ataxia is a rarer form that includes disorders caused by mutations in genes located on the X chromosome. Fragile X-associated tremor/ataxia syndrome (FXTAS) is an example of this type. It arises from CGG repeat expansions in the *FMR1* gene, which generate an expanded RNA molecule with a toxic effect on the cells, leading to neurodegeneration (Raske & Hagerman, 2009).

Mitochondrial mutations or nuclear DNA mutations that affect mitochondria can also lead to ataxia (Ryzhkova et al., 2018). These mutations cause impaired energy production that affects highly mitochondrial ATP-dependent neurons.

The term idiopathic ataxia is reserved for those cases in which no underlying cause can be found after adequate investigation. The genetic basis of this type is unknown and idiopathic ataxias are often a diagnosis of exclusion. Ongoing research is looking for links to mutations in ataxia-related genes in order to help understand the genetic basis of this type (De Silva et al., 2019).

Clinical Progression

The clinical progression of ataxia greatly depends on its etiology.

Early-onset ataxias (e.g. Friedrich's Ataxia) appear during childhood or adolescence and are often a result of genetic factors. Gait instability and loss of coordination are the initial signs, but there is progressive involvement of the upper limbs, speech, and swallowing with the advance of the disorder, leading to great motor dysfunction and disability (Brandsma et al., 2019). Figure 1 shows the most common symptoms encountered by ataxia patients. For most people, symptoms will evolve, with a gradual development of muscle weakness, spasticity, and sensory deficits. This negatively impacts the daily life activities and quality of life of patients, requiring assistive care.

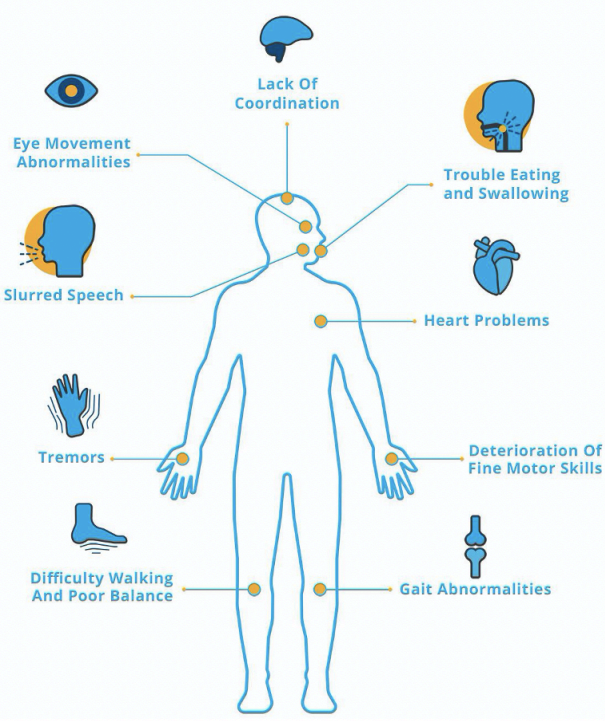

Figure 1. Ataxia Symptoms. Adapted from the National Ataxia Foundation

In contrast, late-onset ataxias manifest during adult life and may be due to genetic factors, such as hereditary mutations, as well as acquired causes like stroke, brain injuries, or autoimmune disorders. The evolution of this kind of ataxia is usually slow compared to the ataxia that begins in childhood (Bhidayasiri et al., 2005). The first signs are loss of balance and unsteady walking, and it evolves into progressive motor dysfunctions, characterized by tremors, difficulty speaking (dysarthria), and loss of coordination in limbs (Dominik et al., 2021). Over time, patients who have such conditions find it very hard to perform daily activities and face a great risk of falling and injury.

The natural course of both early- and late-onset ataxias is progressive, leading to profound impairment and loss of independence. General management and support are of imperative importance in the comprehensive management of the disorder.

Genetic and Molecular Basis of Ataxia

The genetic and molecular basis of ataxia, however, are quite diverse and reflect a wide variety of genetic mutations and pathological mechanisms that can cause the illness.

A very large majority of the genetic ataxias, particularly SCAs, and FA, are caused by trinucleotide repeat expansions. The expansions result in improperly long sequences of a specific trinucleotide, for example, CAG or GAA, causing protein misfolding and aggregation, RNA toxicity, or transcriptional dysregulation (Silveira et al., 2002). Specifically, it is the cerebellum and brain stem that are most affected by this.

For instance, many SCAs are caused by CAG repeat expansions in different genes. This leads to long polyglutamine tracts in the proteins, like ataxin-1 in SCA1, ataxin-2 in SCA2, and also ataxin-3 in SCA3. Protein misfolding and aggregation are other common pathological features in SCAs. The polyglutamine tracts give rise to toxic protein aggregates that compromise cellular functions, including protein degradation pathways, such as the ubiquitin-proteasome system, and mitochondrial function (Minakawa & Nagai, 2021). Sequestration of essential cellular proteins by the aggregates may lead to disruption of cellular homeostasis and triggering of apoptotic pathways (L. Wang et al., 2021).

Mitochondrial dysfunction is another critical event involved in the pathogenesis of ataxias. In FA, the expansion of the GAA repeat in the *FXN* gene leads to lowered expression of the protein frataxin which has a function importantly related to maintaining mitochondrial iron homeostasis. Frataxin deficiencies finally lead to iron-sulfur cluster biogenesis. This effect gives rise to impaired mitochondrial respiration and increased production of reactive oxygen species (ROS)

(Smith & Kosman, 2024). Accumulation of ROS leads to damage of cellular components due to oxidative processes that might add to progressive neurodegeneration in ataxia.

In some ataxias, the disease process involves a pathogenic mechanism of RNA toxicity. Expanded CAG repeats within the *ATXN2* gene, found in SCA2, form toxic RNA foci that sequester specific RNA-binding proteins, interfere with their normal functions, and result in neuronal dysfunction and death (Swinnen et al., 2020).

Non-Coding Repeat Expansions

Pathogenic Mechanisms of Repeat Expansions

Sequences of repetitive non-coding DNA may deeply affect gene expression and function. This type of expansion is associated mainly with neurological and neuromuscular diseases and the mechanisms through which they lead to pathogenicity mainly end in the disruption of different cellular processes.

Non-coding repeat expansions frequently result in transcriptional disruption of the neighboring genes by the formation of abnormal RNA structures that stall the transcriptional machinery (Yanovsky-Dagan, 2015). Repeat expansions can generate very stable hairpins, stem loops, or other secondary structures (Fig. 2B), which stop RNA polymerase and decrease the expression of proteins needed for cell viability and function (Yanovsky-Dagan, 2015). These secondary structures have been documented to partially or completely inactivate gene function, making cells dysfunctional and eventually leading to disease.

Another mechanism is RNA toxicity, which can form RNA foci and then sequester RNA-binding proteins (Fig. 2A). The secondary structures formed by the repeat expansions attract proteins involved in RNA binding, such as heterogeneous nuclear ribonucleoprotein, ubiquitin proteins, or proteasomal subunits (Yanovsky-Dagan, 2015). This, in turn, results in widespread dysregulation of gene expressions since its normal functions involving the splicing, transport, and translation of RNA are disrupted. This misregulation may lead to the accumulation of aberrant transcripts (Fig. 2D) responsible for toxicity and pathology in the cell (Yanovsky-Dagan, 2015). Additionally, the repeat expansions can lead to epigenetic alterations as well. These include DNA methylation and histone modification. Expanded repeats in promotors may recruit methylating enzymes, resulting in hypermethylation and, hence transcriptional silencing of the gene (Chandler et al., 2003). This holds true for conditions in which the repeat expansion involves the promoter or intronic regions of genes leading to transcriptional repression of the affected gene (Figueiredo et al., 2023).

In some cases, non-coding repeat expansions are translated into aberrant proteins through a mechanism called repeat-associated non-ATG initiation (RAN translation) (Yanovsky-Dagan, 2015). Translation of the expanded repeats in alternative reading frames generates toxic polymeric proteins. These aggregation-prone proteins form inclusions that produce cellular stress, disrupt homeostasis, and likely participate in the neurodegenerative process (Yanovsky-Dagan, 2015). Additionally, the proteins produced by RAN translation are believed to directly interact with nucleoporins, disrupting nucleopore formation and function, as well as nucleocytoplasmic transport (Solomon et al., 2021). In turn, dysfunctional nucleocytoplasmic transport can lead to the mislocalization of important proteins in the cell.

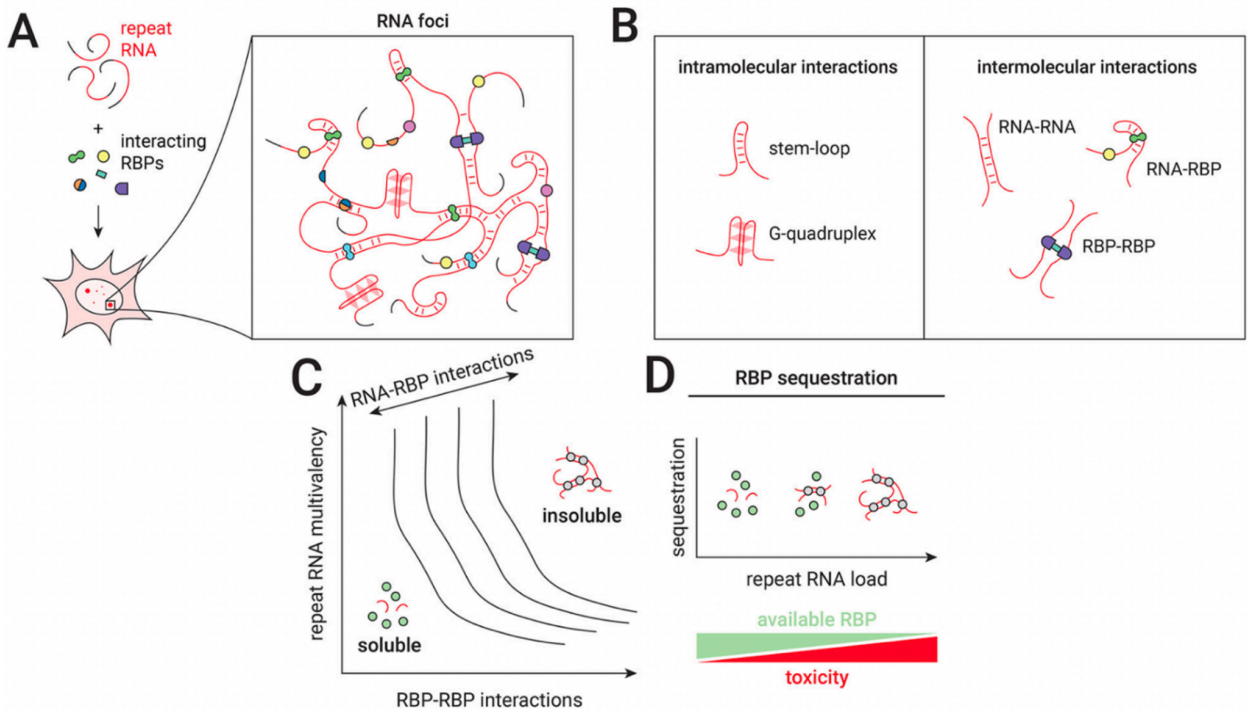

Figure 2. Mechanisms of repeat expansion pathogenesis. Adapted from Malik et al. (2021)

Examples of diseases caused by non-coding repeat expansions

Multiple neurodegenerative diseases besides ataxias have been linked to non-coding repeat expansions.

For example, a large expansion of CGG repeats within the 5'UTR of the *FMR1* gene gives rise to fragile X syndrome (FXS) (Willemsen et al., 2011). In FXS patients, CGG repeats have a size greater than 200 copies, and this leads to hypermethylation of the *FMR1* promoter (Willemsen et al., 2011). Such an epigenetic change causes gene silencing and, as a result, causes suppression of the synthesis of fragile X mental retardation protein (FMRP). This protein has a

vital role in synapses as it ensures proper synaptic function and plasticity. The absence of FMRP disrupts communication within the brain which causes behavioral abnormalities and cognitive deficits seen in FXS (H. Wang, 2015).

Another well-studied example is *C9orf72*-Related Amyotrophic Lateral Sclerosis/Frontotemporal Dementia (ALS/FTD). The GGGGCC (G4C2) repeat expansion in the intron of the *C9orf72* gene constitutes the most frequent cause of ALS and FTD (Yang et al., 2020). The C9orf72 protein is implicated in multiple cellular processes, such as lysosome trafficking, signaling, and autophagy, and is most abundantly expressed in neurons (Smeyers et al., 2021). These repeating expansions create several pathogenic mechanisms: first, the expanded repeats form RNA foci that sequester splicing and RNA-binding proteins, disrupting normal RNA metabolism and producing RNA gain-of-function toxicity (Fujino & Nagai, 2022). The RAN translation of G4C2 repeats generates dipeptide repeat proteins, which aggregate and form intracellular inclusions that contribute to cellular toxicity (Rodriguez & Todd, 2019). Finally, toxic aggregates disrupt many cellular processes, such as nucleocytoplasmic transport, leading to the dysfunction and death of neurons.

FGF14 and Ataxia

FGF14 Gene Structure and Protein Function

FGF14 is part of the FGF family. Members of this family have important functions in a variety of biological processes, most importantly in the development and function of the nervous system (L. Wang et al., 2021).

The *FGF14* gene is located on the long arm of chromosome 13, 13q34, in humans. It consists of three exons and translates into a peptide of approximately 24 kDa in size. *FGF14* is a member of the iFGF subfamily, which can not be secreted and doesn't interact with FGF receptors, hence, remains intracellular (Laezza et al., 2009).

FGF14 is expressed the most in the central nervous system (CNS) and is required for normal neuronal function. The highest levels of expression are in the cerebellum, hippocampus, and cortex, regions important for motor control, learning, and memory. In the nervous system, FGF14 excerpts its function through interactions with voltage-gated sodium (Nav) channels, such as the subtypes Nav1.2 and Nav1.6 (Laezza et al., 2009).

FGF14 binds directly to the intracellular C-terminal domains of Nav channels, where it affects their gating properties and kinetics (Laezza et al., 2009). FGF14 influences the proper localization of the Nav channels to the sites involved in action potential initiation and

propagation, such as the axon initial segment (AIS) and the nodes of Ranvier (Laezza et al., 2009). Modulation of these channels by FGF14 is, therefore, a critical regulator of neuronal excitability and synaptic transmission.

FGF14 also plays a modulating role with respect to synaptic plasticity, the cellular basis of learning and memory. It does so by influencing activity-dependent Nav channel trafficking that, in turn, influences the strength and effectiveness of synaptic contacts (Alshammari et al., 2016). Furthermore, FGF14 is involved in the developmental processes of neurons. It contributes to the maturation and stabilization of neuronal circuits and a loss of *FGF14* function can lead to an accumulation of immature neurons (Alshammari et al., 2016). Disruptions in *FGF14* expression or function caused by repeat expansions or single nucleotide mutations can lead to significant neurodevelopmental abnormalities and have been associated with a variety of neurological or neurodegenerative disorders like ataxia, depression, anxiety, and schizophrenia (Di Re et al., 2017).

More recently, the non-coding repeat expansions of the *FGF14* gene were linked to SCA27, a type of ataxia characterized by progressive motor incoordination and dysfunction of the cerebellum (Pellerin et al., 2023). It has been shown that *FGF14* mutations alter the normal interactions between FGF14 and the Nav channels, eventually leading to disrupted motor control functions (Laezza et al., 2009).

Link Between *FGF14* and Ataxia

Genetic studies have greatly expanded our understanding of many neurodegenerative diseases, such as SCA27 and late-onset cerebellar ataxia (LOCA), in which *FGF14* mutations are involved. Several research studies on the *FGF14* gene discovered that heterozygous GAA trinucleotide repeat expansions in its first intron were associated with SCA27 and LOCA - progressive motor disorders beginning in adulthood (Rafehi et al., 2023).

Strong evidence linking these GAA repeat expansions with the pathogenesis of ataxia was demonstrated by Pellerin et al. (2024). Their work showed that these genetic mutations disrupt the expression level of *FGF14*, with the characteristic symptoms of ataxia - loss of coordination and balance.

The same study by Pellerin et al. (2024) found that these expansions were common in patients with ataxia syndromes, peripheral neuropathy, and bilateral vestibulopathy. All of these conditions are associated with impaired balance and control of the limbs.

The authors screened 45 cases of cerebellar ataxia and identified a heterozygous *FGF14* GAA repeat expansion of ≥250 repeats in 38% of the cases, thereby definitively establishing the involvement of this gene in ataxia etiology.

Evidence also indicates that the pathogenicity threshold likely lies in the range of 250-300 GAA repeats (Pellerin et al., 2024). There is also a direct correlation between longer expansions and more severe phenotypes, as well as an inverse correlation between the length of the expansion and onset age (Bonnet et al., 2023). The frequency at which this repeat expansion is identified in patients with neurodegenerative disorders proves the role played by *FGF14* in maintaining normal cerebellar function, thus being a potential therapeutic target.

Moreover, Pellerin et al. (2023) also discovered that the expansions of the *FGF14* GAA repeats are a common genetic LOCA cause across different population groups, which included French Canadian, German, Australian, and Indian cohorts.

The exact mechanisms by which GAA repeat expansions in *FGF14* lead to ataxia are still being investigated. Several plausible mechanisms have been proposed in recent studies. One prominent possibility is that the repeat expansion leads to haploinsufficiency, where the mutated allele produces low levels of FGF14 protein (Pellerin et al., 2023).

Moreover, it is suggested that the expansion can also lead to transcriptional interference, where the aberrant RNA is unable to be translated into a functional protein, also leading to low levels of FGF14 protein (Rafehi et al., 2023).

Additionally, point mutations can happen concurrently with repeat expansions (Mohren et al., 2024). The combination of the abnormal protein due to the repeat expansions, together with the point mutations can further compromise protein function.

Another important point to consider in the pathogenesis of this disease is the increase of repeat size over generations. It is thought that GAA expansions primarily increase through maternal transmission, while paternal transmission leads to a contraction of repeat size (Bonnet et al., 2023; Ouyang et al., 2024). It is believed that the *FGF14* repeat expansions are highly unsatiable upon meiotic transmission leading to increased repeat size transmitted from the mother (Bonnet et al., 2023).

These mechanisms of transcriptional interference, haploinsufficiency, increase in expansion size, as well as concurrency with other point mutations, likely contribute together to the neurodegeneration seen in ataxia patients.

Cellular and Molecular Impact

The GAA repeat expansions in *FGF14* cause substantial downregulation of the gene. This downregulation produces a loss of normal function of FGF14, which is a potent modulator of voltage-gated sodium channels inside neurons (Laezza et al., 2009). The dysfunction of these channels leads to the severe motor deficits seen in ataxia. Studies by Pellerin et al. (2023) and many others have shown that patients with these expansions had significantly lower levels of FGF14 RNA and protein expression compared to controls (Fig. 3). The loss of FGF14 impairs this interaction, rendering sodium channels dysfunctional, and is linked to altered impulse generation and propagation, due to the channel's localization in the AIS and the nodes of Ranvier (Laezza et al., 2009).

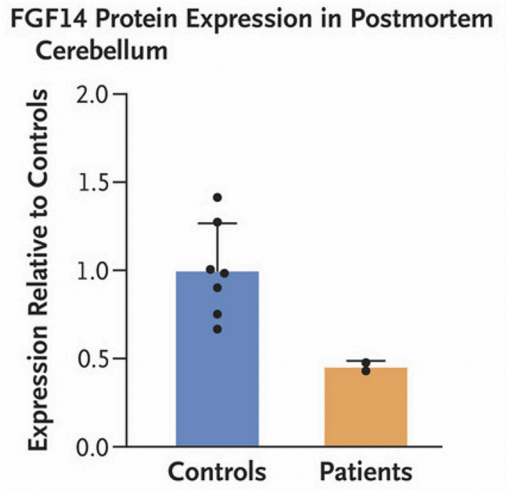

Figure 3. Mean expression ratios of FGF14 protein in postmortem cerebellar specimens from seven controls and two patients. Adapted from Pellerin et al. (2023).

One of the most significant downstream pathways influenced by the mutations in *FGF14* is the regulation of intracellular calcium levels. FGF14 is believed to be involved in the modulation of multiple calcium channels, and calcium signaling in the absence of FGF14 is altered in neurons (Yan et al., 2013). This, in turn, will impact several other cellular processes, like neurotransmitter release and neuronal survival.

Furthermore, FGF14 regulates synaptic plasticity, a cellular process forming the basis for learning and memory. It plays an important role in the trafficking of activity-dependent Nav channels, that have the role of strengthening synaptic connectivity and efficacy (Alshammari et al., 2016). Reduced FGF14, through the dysregulation of such activity-dependent trafficking, could be a contributor to deficits in synaptic plasticity associated with ataxias leading to dysfunctional cognition and motor activity.

Additionally, FGF14 deficiency also activates cellular stress response pathways. The potential accumulation of misfolded proteins or RNA and loss of cellular homeostasis can activate cellular stress pathways, such as the production of ROS or apoptosis, that might exaggerate damage to neurons and exacerbate the progressive neurodegeneration in ataxia (L. Wang et al., 2021).

Phenotypic Consequences

Behavioral and neurological testing in animal models and human studies has greatly improved our understanding of the phenotypic effects of *FGF14*-related ataxia. A phenotype resembling

SCA27 was observed in *FGF14* deficient mice, with coordination and motor problems (Bosch et al., 2015).

Neurological exams on individuals suffering from SCA27 reveal a gradual loss or progressive deterioration of motor skills and functions. Early symptoms include loss of balance, unsteady gait, and difficulty with fine motor tasks. Even though SCA27 seems to progress slower than other SCAs, severe motor disability symptoms, dysarthria, difficulty in swallowing (dysphagia), and tremors can also appear over time (Groth & Berman, 2018).

Imaging and neuropathological examination, represented in Figure 4, of affected individuals have shown high rates of neurodegeneration, especially in the cerebellar vermis (Pellerin et al., 2023). Another study by Chen et al. (20214) has reported atrophy of the vermis as a characteristic feature in 74-97% of all patients with GAA-*FGF14* ataxia.

The death of the Purkinje cells is extremely significant since they are responsible for motor coordination. Apart from the Purkinje cells, other types of neurons and structures of the brain could be involved in causing motor deficits in ataxia. For example, the excitatory input of the granule cells and the inferior olivary neurons onto Purkinje cells is believed to be impaired in SCA27 (Chopra & Shakkottai, 2014; Tempia et al., 2015).

Electrophysiological recordings from neurons in animal models with *FGF14* mutations reveal altered spiking and defects in synaptic transmission (Tempia et al., 2015). Again, these findings suggest that *FGF14* is required for the maintenance of appropriate neuronal excitability and neurotransmission. The disruption of these processes due to reduced FGF14 advocates for the proposed molecular mechanisms underlying the motor disability observed in ataxia.

Expansions of GAA repeats in *FGF14* hence can be assumed to cause neurodegeneration and particularly atrophy of the cerebellum. Ataxic symptoms perhaps result from damage or degeneration of highly *FGF14*-expression-dependent Purkinje neurons of the cerebellum.

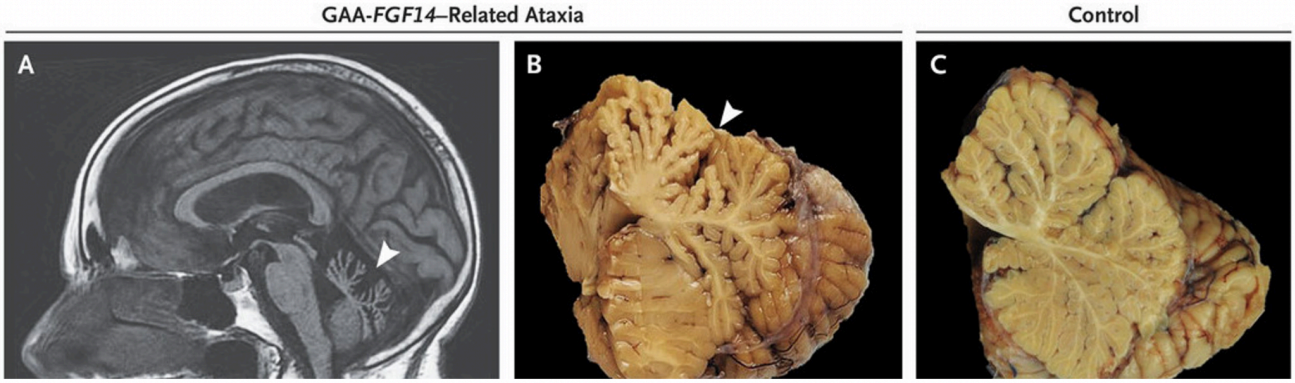

Figure 4. Imaging and Neuropathological Findings in Patients with GAA-FGF14–Related Ataxia. A) Severe vermis atrophy (arrowhead) on sagittal T1-weighted magnetic resonance imaging in a female patient at 88 years of age. B) Midsagittal section of the postmortem cerebellum of the same patient at 94 years of age, in which anterior vermis atrophy is visible (arrowhead); C) Age-matched control for comparison. Adapted from Pellerin et al. (2023).

Discussion and Conclusion

Interpretation of Findings

The currently available literature reveals significant insights into the role of *FGF14* in ataxia, particularly spinocerebellar ataxia type 27. The discovery of intronic GAA repeat expansions in *FGF14* as a cause of SCA27 has provided a definitive diagnosis for many patients previously categorized under idiopathic ataxia. This genetic mutation's identification has clarified the etiology for over 30% of such cases, offering a new perspective on diagnosis and management (Van De Warrenburg & Kamsteeg, 2024).

FGF14 is a critical protein for neuronal function, particularly in controlling voltage-gated sodium channels for neuronal excitability and synaptic transmission. Repeat GAA expansions introduce disruptions of the normal functioning of the *FGF14* gene with reduced expression and impaired trafficking of Nav channels (Laezza et al., 2009). This leads progressively to motor dysfunction, loss of coordination and balance, and other ataxia symptoms. The impact of repeat expansion on the expression and function of FGF14 highlights its general importance for the maintenance of cerebellar function and neuronal signaling pathways.

The major conclusions from the existing studies confirm that GAA repeats are pathogenic in *FGF14*, where expansions at the critical range of 250-300 are especially detrimental in their effect (Bonnet et al., 2023). The widespread identification of these expansions across different populations further puts a seal of universality on this genetic mutation and its role in ataxia pathogenesis.

Mechanistic Insights

Several mechanisms may be involved in GAA repeat expansions within *FGF14* that lead to ataxia.

One key mechanism believed to be involved is transcriptional interference when the abnormally long RNA molecules can form structures that sequester RNA-binding proteins, hence disrupting RNA transcription (Rafehi et al., 2023). Such aberrant RNA structures can also perturb the splicing of *FGF14* transcripts, further lowering the levels of functional FGF14 protein.

Another critical mechanism is the haploinsufficiency hypothesis, where the mutated allele is unable to produce sufficient levels of functional FGF14 protein (Pellerin et al., 2023).

Additionally, *FGF14* loss impairs Nav channel function at the cellular level, which in turn disrupts neuronal excitability and synaptic transmission at the brain level (Laezza et al., 2009). Impaired

calcium signaling, a downstream pathway affected by the disrupted Nav channel function, negatively impacts neurotransmitter release and neuronal survival, hence contributing to ataxia (Yan et al., 2013).

Current Gaps in Knowledge

While it is established that GAA repeat expansions in *FGF14* lead to reduced expression of the FGF14 protein, the precise mechanisms whereby this causes neuronal dysfunction and degeneration are not fully understood.

FGF14 is known to interact with and functionally regulate Nav channels, especially Nav1.2 and Nav1.6, in Purkinje neurons (Laezza et al., 2009). The current thinking is that reduced levels of FGF14 impair cell surface expression and localization of such channels, therefore altering neuronal excitability and firing patterns (Laezza et al., 2009). The exact molecular events have yet to be fully understood. Other types of channels are possibly also involved in the disease pathogenesis, but current literature is limited.

The primary pathology in SCA27 appears to be Purkinje neuron dysfunction since they are the sole output of the cerebellar cortex. However, how this exerts downstream effects on other cerebellar circuits, or whether other cell types contribute, for example, granule cells or inferior olivary neurons, is not understood (Consalez et al., 2021).

While SCA27 is considered to be an ataxia type solely involved in cerebellar dysfunction, more recent studies have described additional non-cerebellar pathologies mainly in the cognitive and neuropsychiatric areas (Di Re et al., 2017). Little is known of mechanisms responsible for these non-motor symptoms, and whether brain regions other than the cerebellum could be influenced by *FGF14* repeat expansions.

In SCA27, the genetic landscape is particularly complex, and a rather substantial variation in size of the GAA repeat expansions and their associated phenotypes has been described. While expansions of ≥ 250 GAA repeats are usually considered pathogenic, the clinical significance of intermediate-sized expansions (<250 repeats) is less clear. Some heterozygotes for these intermediate expansions can show mild phenotypes, while others may have severe symptoms, similar to the greater expansion length (Bonnet et al., 2023). In the latter case, co-occurring pathogenic variants in other ataxia genes could also be involved in the development of the severe phenotype. Single point mutations in the *FGF14* gene cooccurring with the repeat expansions can also contribute to a wider range of clinical presentations and can influence the severity, rate of progression, and age of onset of the disease. Unfortunately, current literature

lacks detailed information on these two types of mutations happening concurrently in the same patient.

Relatively consistent disease phenotype and mechanism are associated with *FGF14* GAA repeat expansion across the diversity of populations studied (Pellerin et al., 2023). A more extended investigation would be required to include individuals from a greater range of ethnic backgrounds in order to examine the geographic variations in disease presentation or repeat characteristics.

The meiotic instability of *FGF14* repeat expansions also contributes to the complexity of the disease. While it is believed that repeat expansions increase in size through maternal transmission due to somatic instability, more information is needed to elucidate the mechanism of expansion, as well as the factors influencing it. Additionally, more information is needed to map the behavior of the expansions over generations across different populations to understand the mechanisms of long-term stability.

In summary, while the discovery of *FGF14* repeat expansions has unraveled a significant part of the genetics underlying ataxia, several gaps in knowledge on precise mechanisms of pathogenicity, genetic heterogeneity, and possible contribution of non-cerebellar pathways still exist (Fig. 5). Further research to fill these gaps will be crucial in formulating an effective diagnostic strategy and potential therapeutic interventions for SCA27 and related ataxias.

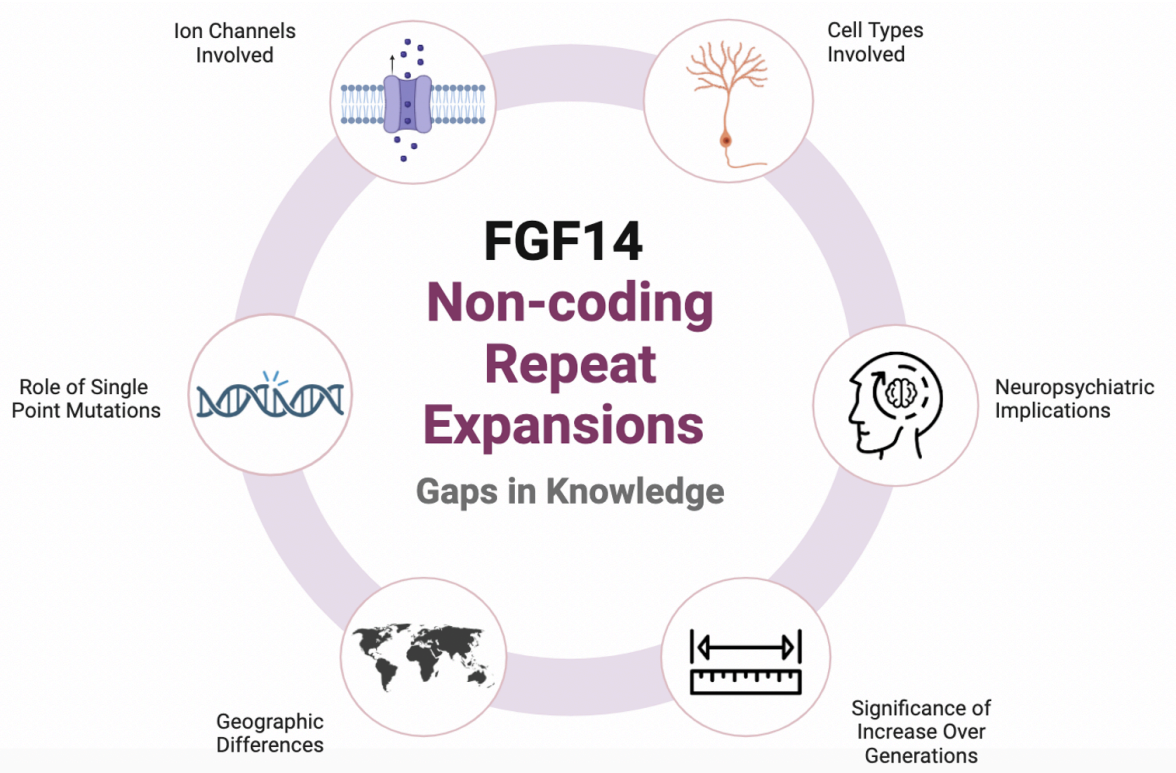

Figure 5. Gaps in knowledge in current understanding of *FGF14* repeat expansion pathogenesis. Created using BioRender

Future Directions

Future studies can be directed toward determining more precisely the molecular mechanisms of *FGF14*-related ataxia. State-of-the-art functional genomic and transcriptomic studies could identify misregulated pathways by GAA repeat expansions and provide potential therapeutic targets. Investigations into RNA-binding proteins and their sequestration by expanded RNA repeats may reveal additional ways of improving transcription.

Gene therapy or RNA-targeted therapies that could either restore normal FGF14 function or reduce aberrant RNA species are promising in this direction. Specifically, expanded repeats could be targeted and modified using methods like CRISPR-Cas9 genome editing or antisense oligonucleotides, reversing or mitigating the phenotype of the disease (Hwang et al., 2024).

Phenotypic variability of SCA27 patients was observed even in individuals bearing the same repeat expansion (Groth & Berman, 2018). Because of that, genetic modifiers could be involved. The identification of such modifiers by genome-wide association studies or next-generation sequencing might unravel further mechanisms of the disease or therapeutic targets.

Future research directions in the field of *FGF14* and ataxia should focus on addressing the remaining gaps in our understanding of the disease mechanisms and genetic landscape.

Although the interaction of FGF14 with Nav channels has been recognized, the precise molecular pathways of neuronal dysfunction and degeneration remain to be determined. This may be established by the study of ion channel trafficking, localization, and function in the absence of FGF14, as well as possible downstream consequences on cerebellar circuitry and other brain regions.

Although SCA27 can still be considered predominately a type of cerebellar ataxia, non-motor symptoms also play a crucial role in the disease burden. A study on the possible involvement of *FGF14* in non-cerebellar brain regions and the mechanisms through which it operates is therefore relevant for broadening the understanding of the clinical spectrum of SCA27.

This would require a strong set of biomarkers and outcome measures, that would permit the follow-up of disease progression and the efficacy of any possible therapeutic interventions.

Future studies on the mechanisms underlying the disease should be focused on potential therapeutic strategies for SCA27 by modulating *FGF14* expression, targeting downstream pathways, or gene therapy/RNA-based therapy to correct the genetic defect.

Conclusion

Finally, research into the role of *FGF14* in ataxia, particularly SCA27, has gone a long way to improve our understanding of this neurodegenerative disorder. Identification of intronic GAA repeat expansions in the *FGF14* gene as a definitive cause of SCA27 explained many cases previously labeled as idiopathic ataxia. These results demonstrate the need for FGF14 in normal neuronal function, particularly in regulating voltage-gated sodium channels required for neuronal excitability and synaptic transmission.

The mechanisms underlying *FGF14*-mediated ataxia are very heterogeneous, including disruptions in RNA transcription, haploinsufficiency, RNA toxicity, and epigenetic modifications. Together, these mechanisms give rise to neurodegeneration in SCA27. More precisely, GAA repeat expansions in *FGF14* lower the expression of functional FGF14 protein that impairs sodium channel function, which affects neuronal excitability and probably leads to synaptic dysfunction.

In spite of these advances, there is much that remains unknown. For example, the molecular pathways by which *FGF14* dysfunction leads to neuronal degeneration have not yet been fully described. Furthermore, phenotypic expression is rather variable among cases with identical mutations, suggesting there must be other genetic modifiers at work that have not yet been identified. In addition, it has not been assessed up until now to what degree non-cerebellar brain areas are actively contributing to the clinical manifestations of SCA27.

Future studies should be aimed at understanding the molecular mechanisms underlying *FGF14*-related ataxias. Comprehensive genomic and transcriptomic investigations could unravel misregulated pathways and identify therapeutic targets. Gene therapies or RNA-targeted therapies, may prove to be effective in the restoration of normal *FGF14* function. Longitudinal studies and clinical trials can be performed to evaluate the efficacy of any potential treatments.

These research gaps are critical to be addressed in order to provide effective diagnostic tools and therapeutic strategies for SCA27 and related ataxias. Further research on the genetic, molecular, and cellular mechanisms of *FGF14*-related ataxia will help bring us closer to the treatment of this debilitating disorder.

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