Article title: The quest towards understanding the molecular pathogenesis of triplet repeat disorders: Huntingtons Disease and Fragile X-Associated Tremor and Ataxia Syndrome

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The quest towards understanding the molecular pathogenesis of triplet repeat disorders: Huntington's Disease and Fragile X-Associated Tremor and Ataxia Syndrome

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Authors summary:

Trinucleotide repeat disorders (TRDs) make up a group of neurological inherited diseases mediated by unstable repeat expansions. Polyglutamine expansion leads to diseases such as Huntington's Disease (HD), while polyglycine expansions lead to diseases such as Fragile-X Associated Tremor and Ataxia Syndrome (FXTAS). The normal huntingtin gene contains 6-34 CAG repeats; however, upon a threshold effect that exceeds 36 repeats, HD becomes fully penetrant and promotes degeneration of neuronal populations. In contrast, FXTAS arises from polyglycine repeats between 22-200. FXTAS carriers experience severe tremor, ataxia and brain atrophy. Here, we analyze the various ways mutant huntingtin and *FMR1* mRNA aggregates induce intracellular dysfunction in HD and FXTAS, specifically in the context of impaired neuronal processes and the protein-protein interactions. The identification of these molecular processes offers potential targets for drug intervention and could lead to the development of innovative therapies to treat HD and FXTAS neuropathogenesis. (145)

Abstract

Trinucleotide repeat disorders encompass a group of neurological diseases driven by unstable repeat expansions. Huntington's disease (HD) is characterized by chorea and brain atrophy. The normal huntingtin protein contains 6-34 CAG repeats; however, upon a threshold effect of >36 repeats, the huntingtin protein acquires toxic mechanisms that are harmful to the cell. Fragile X-Associated Tremor and Ataxia (FXTAS) is characterized by tremor, ataxia and neuronal loss. The normal *FMR1* gene contains 5-45 CGG repeats; however, upon a "premutation" CGG expansion of 55-200, FXTAS arises. A pathological hallmark of these disorders includes aggregate formation within the brain; suggestive of impaired protein and mRNA function. Furthermore, important molecules have been found to be sequestered into these aggregates withdrawing them from their normal roles. In this review, the ways mutant huntingtin and *FMR1* mRNA aggregates induce intracellular dysfunction in HD and FXTAS is analyzed, specifically in the context of impaired neuronal processes and protein-protein interactions. Analysis revealed that huntingtin and *FMR1* mRNA are involved in the regulation of multiple cellular pathways; and whose impaired function results in a detrimental domino-effect that is destructive to the cell. The understanding of these molecular processes hopes to identify potential targets towards the treatment of HD and FXTAS. (200)

Trinucleotide repeat disorders

Introduction

Trinucleotide repeat disorders (TRDs) encompass neurological inherited diseases mediated by unstable repeat expansions (Figure 1). TRDs are classified into coding and noncoding disorders (1). Coding disorders include polyglutamine expansion, such as Huntington's Disease (HD). Noncoding disorders include polyglycine expansions, such as Fragile-X-Associated Tremor and Ataxia Syndrome (FXTAS).

HD is an autosomal dominant disorder induced by CAG expansion located within the open-reading frame of exon 1 of the *HTT* gene (2). The normal huntingtin gene contains 6-34 CAG repeats; however, upon a threshold effect that exceeds 36 repeats, HD becomes fully penetrant and promotes neuronal degeneration, this induces chorea and progressive cognitive deficits (2). Although the function of wild-type (WT) huntingtin (Htt) is unknown, huntingtin is expressed within the brain and interacts with structures such as the mitochondria and microtubules (3). There is no treatment for HD and death occurs within 10-15 years. HD age-of-onset inversely correlates with CAG length; however, individuals with longer repeats can experience earlier age-of-onset (2). CAG repeats >28 experience greater instability upon replication which promotes polyglutamine expansion. This accounts for anticipation (4), in which age-of-onset occurs earlier in successive generations.

FXTAS is caused by a "premutation" CGG expansion located within the 5'untranslated region of the *FMR1* gene (5). The normal *FMR1* gene contains between 5-45 CGG repeats; however, when the polyglycine expansion is between 55-200, it becomes pathogenic (5). In contrast, CGG expansions >200 result in full gene mutation and lead to Fragile-X-Syndrome. FXTAS is characterized by tremor, gait ataxia, cognitive deficits and brain atrophy (5). As with HD, FXTAS age-of-onset inversely correlates with increased CGG repeats. Furthermore, CGG repeats express anticipation (6). Although the WT function of *FMR1* gene protein (FMRP) is unknown, it is thought to be involved in synapse development and translation (5).

A neuropathological hallmark of HD and FXTAS includes the formation of protein and mRNA inclusion bodies (IBs), respectively, within the brain (2,5). At the molecular level, CAG

expansions and *FMR1* CGG premutation carriers express a 2-8-fold increase in huntingtin and *FMR1*mRNA, respectively (7,8). Although the role of IBs in HD and FXTAS is unknown, various proteins and RNAs have been found sequestered into the IBs. In this review, the mechanism of aggregate formation is briefly reviewed. The ways mutant huntingtin (mHtt) and *FMR1*mRNA aggregates induce intracellular dysfunction in HD and FXTAS is analyzed, specifically in the context of impaired neuronal processes and the protein-protein interactions within the proteostasis network. The identification of these molecular processes offers targets for drug intervention and therapy development to treat HD and FXTAS.

Methods

PubMed and Google Scholar databases were used to retrieve articles using keywords relevant to the molecular pathogenesis and neuropathology of HD and FXTAS (e.g.,

“Huntington’s Disease” AND/OR “molecular pathology” AND/OR “neuropathology” AND/OR “therapeutics” the same was applied for FXTAS). Inclusion criteria: peer-review and primary literature. Database filters were applied to include articles released after the 1990s due to the pioneering work performed on these diseases.

Mechanisms of Aggregate Formation

The polar zipper and gain-of-function model

The polar zipper model states that huntingtin conformation is destabilized by expanded polyglutamine tracts, which lead to abnormal mHtt-mHtt interactions (2) (Figure 2). Polyglutamine protein-protein interactions induce the formation of insoluble β -pleated sheets via hydrogen bonding (2). McGowan *et al.* (9) stained IBs of HD brain samples with Congo Red, which resulted in dye polarization, a characteristic state of proteins with β -pleated sheets. Furthermore, a positive correlation was identified between increased repeat size and β -hydrogen bond formation (10). Although this model is an attractive theory, it fails to consider the interactions between CAGs and other proteins. This gain-of-function mechanism might explain some aspects of FXTAS-mediated toxicity, given multiple proteins have been identified to co-localize in mRNA aggregates (Figure 3).

The transglutaminase model

Transglutaminases (TGs) are enzymes that mediate the crosslinking of glutamine-glutamine residues via covalent ϵ -lysine residues (2) (Figure 2). Cortex HD brain sample analyses revealed that 99% of TG-2 activity co-localized with mHtt (11). Polyglutamine tracts might increase glutamine-transglutaminase crosslinking activity, resulting in IBs. The transglutaminase model is an appealing theory for aggregate formation; however, some data contradict this model. Huntingtin IBs might also result from TG-mediated polyamination (12). In support of this, 1mM of putrescine was seen to almost inhibit tau crosslinking and given polyamines are found within the millimolar range (12), thus; mHtt-polyamination cannot be ruled out.

Repeat associated non-ATG translation (RAN) model

The repeat associated non-ATG translation (RAN) model describes a non-ATG mediated 5'UTR *FMR1* translation of toxic proteins (5) (Figure 3). RAN-based CGG translation occurs in two *FMR1* transcript reading frames. FMRpolyG is the main RNA translation product, while FMRpolyA is the second (5). FXTAS brain sample analyses identified FMRpolyG/A-nuclear aggregates that cosegregate with ubiquitin- and heat-shock-positive inclusions (13,14). The role RAN-based translation products play in FXTAS pathogenesis is unknown; however, protein co-localization within aggregates suggests that these products interact with proteins whose normal function is impaired upon sequestration. The RAN translation model displays a different gain-of-function model, which might mediate FXTAS pathogenicity.

Figure 3. Schematic representation of RNA-gain of function model and RAN-based translation model. A. RNA-gain of function model displays how proteins can be sequestered by binding to polyglycine repeats directly or indirectly (e.g. Pura-Rm62). This depletes bound proteins from their protein pools, decreasing their interactions with target substrates and activating a downstream effect of impaired pathways. B. Repeat associated non-ATG translation (RAN) model. The polyglycine repeat is believed to induce the formation of a hairpin structure, as the translation initiation complex binds it stalls due to the hair-pin, promoting non-ATG based translation with similar start codons (CTG), this results in a frame-shift producing FMRpolyG, FMRpolyA or rarely FMRpolyR, these proteins can then sequester proteins in a similar model as RNA-gain of function seen in (A) and in HD. Figure adapted from (52).

Intracellular dysfunction in HD and FXTAS

Polyglutamine and polyglycine expansion in HD and FXTAS, respectively, is hypothesized to result in a gain-of-function mechanism that is toxic to the cell. In contrast, loss of wild-type function disrupts homeostasis. The contribution of protein and mRNA toxicity to the deregulation of intracellular pathways is analyzed.

Huntington's disease, heat-shock proteins and transcription factors

Huntingtin aggregation is polyglutamine length-dependant and co-localizes with heat-shock proteins (HSPs). HSPs are chaperone proteins that prevent protein misfolding and aggregation (2). Transfection of three different cell lines with GFP-mHtt of various polyQ lengths induced aggregate formation and HSP co-localization across all cell lines (15). Apart

from HSP-co-localization, analyses of mice neuroblastoma cells(CAG₉₆) and yeast-models(CAG₉₆) identified the co-localization of TATA box binding protein (TBP), a transcription factor (TF) required for protein transcription (15). Schaffar *et al.*, (15) suggested that mHtt-TBF interaction is due to polyglutamine-polyglutamine interactions, resulting in Beta-sheet folding and aggregation. Indeed, live neuronal imaging identified the N'terminus TBP domain PolyQ tract to co-localize with mHtt aggregates (16), suggestive of transglutaminase-mediated aggregation. Furthermore, TBP-associated factor-4(TAF4) was seen to co-localize with mHtt aggregates (17). This is important given TBP-TAF4 comprises transcription factor II D (TF_{II}D), a transcription complex required for PGC-1 α transcription (17). PGC-1 α is a TF that regulates metabolic processes. A PGC-1 α mRNA analysis of presymptomatic HD striatum samples displayed a 30% activity decrease (17). These results indicate that PGC-1 α expression is decreased in the caudate, one of the first regions known to degrade in HD (18). While TAF4 overexpression in mHtt(Q₁₁) striatal cells reversed huntingtin-induced PGC-1 α inhibition (17). These findings indicate that mHtt-mediated TBP-TAF4 sequestration impairs TF_{II}D gene transcription, resulting in alterations of energy metabolism genes. Ultimately, energy and mitochondrial dysfunction increases reactive-oxygen species, leading to neuronal death. Interestingly, a gene-set enrichment analysis of PGC-1 α -KO mice revealed how closely downregulated pathways in the mice matched those from early-stage HD patients (19). These findings suggest that decreased PGC-1 α might represent an early defective step in HD.

Previous studies have suggested that HSP-overexpression decreases huntingtin aggregate formation. For example, HSP40/70 overexpression decreased aggregation with no TBF co-localization (15), which suggests that HSP40/70 interferes with polyQ toxicity and protein-binding. Although HSP overexpression might decrease aggregation, there is contradictory data. For example, proteasome purification of insoluble fractions of an R6/2 HD mouse model identified 1.5% of the content to be HSP40, -70 and -90 (19). There are some possible explanations for the discrepancies between studies. First, different cell lines used in the studies might express different HSPs that alter IB formation (15,20). For example, antagonist interactions between HSP104 and HSP70 suggest that polyQ aggregation depends on their overall balance (20). While transfection of different vectors might impact different set

of genes. Although induced expression of heat-shock factor1 and heat-shock response increased the R6/2 HD mouse model lifespan, aggregate reduction was transient and disappeared during disease progression (21). These findings indicate that throughout disease progression, HSPs attempt to unfold mHtt but become sequestered into IBs, implying that their effects are compensatory.

Huntington's disease, heat-shock proteins and transcription factors

Huntingtin polyglutamine expansions impair the heat-shock response (HSR). HSF1 is a TF involved in the activation of an HSR and HSPs (22). Genome-wide chromatin immunoprecipitation of mHtt(Q₁₁₁) striatal neurons displayed decreased HSF1 activity capable of binding 39% of target genes (22). These results suggest that mHtt fails to activate an HSR. Recent studies have implicated a role for CK2 α' kinase and Fbxw7 (23). CK2 α' kinase and Fbxw7 are proteins that phosphorylate and stimulate ubiquitin-dependent degradation. Medium spiny neurons (MSNs) of HD mice models(Q₁₇₅) and HD patients' striatal tissues exhibited increased CK2 α' and Fbxw7 levels (23). Mass spectrometry analysis revealed that CK2 α' phosphorylates HSF1 at S303 and S307 sites, promoting its degradation. Furthermore, Fbxw7 removal also increased HSF1 levels. Gomez-Pastor *et al.*, (23) identified a mechanism of defective HSF1 activation, given mHtt targets HSF1 for proteasomal degradation via increased CK2 α' and Fbxw7 activity. Interestingly, muscle tissue analyses of HD mice also expressed increased CK2 α' levels (23). Furthermore, late-stage R6/2 HD mice models were seen to undergo cachexia similarly to late-stage HD patients (24). This finding suggests a link between the PNS and CNS that might explain how progression of mHtt IBs and increased CK2 α' levels within muscle tissues might represent a late dysfunction in HD.

Data show that HSP interaction with mHtt occurs as an attempt to prevent aggregation; however, HSPs that undergo huntingtin sequestration result in widespread chaperone-loss, leading to a substantial increase in misfolded proteins.

FXTAS, heat-shock proteins and RNA-binding proteins

FXTAS neuropathology might be mediated by impaired laminA/C and HSPs. LaminsA/C are filament proteins that maintain cell integrity by lining the nuclear membrane. Previous studies have identified the co-localization of α B-crystallin (HSP) and laminA/C within cortex

samples of FXTAS patients (25). Transfection of a FMR(CGG₈₈)-repeat into neuronal progenitor cells resulted in a 6% increase in IB, where 3% and 5% of them positively stained for lamin A/C and α B-crystallin, respectively (26), suggestive of polyglycine-induced α B-crystallin sequestration. Interestingly, as time progressed, the FMR(CGG₈₈) neuronal cells experienced laminA/C nuclear degradation as the lamin ring disappeared. A separate study identified increased mRNA levels of α B-crystallin and HSP27/70 within fibroblast cultures and cortex samples from FXTAS patients (27). A negative correlation between mRNA and protein levels was shown. Surprisingly, analyses of fibroblast cultures from five premutation carriers who had not experienced FXTAS core symptoms displayed no significant differences in mRNA levels (α B-crystallin, HSP27 and HSP70) compared with FXTAS patients (27). Furthermore, premutation mouse embryonic fibroblast (MEF) cells also displayed *FMR1*mRNA-induced laminA/C abnormalities (27). The association between α B-crystallin, HSP27/70 and laminA/C might reflect an early stress-response to polyCGG. This finding is supported by the fact that α B-crystallin was found to elicit a protective function on sarcomeres upon heat-shock (28). The decrease in HSPs and FMRP mRNAs might reflect sequestration into *FMR1* mRNA IBs, inhibiting protein translation. Interestingly, *FMR1*mRNA expression levels were similar across premutation carriers and FXTAS patients. It was surprising how quickly MEF HD models experienced impaired laminA/C, which suggests that CGG repeats trigger laminA/C dysfunction early in FXTAS. The fact that no ubiquitin was detected in the IBs (27), suggests that aggregate formation is independent of the ubiquitin-protease degradation system and that ubiquitin-tagged *FMR1*mRNAs might be a late event in FXTAS. These findings provide useful data for early diagnosis of premutation *FMR1* carriers and a basis for the early neurodevelopmental abnormalities seen in children.

FXTAS, heat-shock proteins and RNA-binding proteins

*FMR1*mRNA-mediated sequestration of Pur α contributes to FXTAS pathogenesis. Pur α is an RNA-binding protein that regulates RNA-functions (5). Pur α KO mice model exhibited a decrease in neuron formation and myelin reduction (29). Although Pur α KO did not induce embryonic lethality, 2-week old mice developed severe tremors, suggesting that Pur α KO can lead to ataxia (29). Cerebral cortex analyses of Pur α KO mice also revealed memory deficits and

decreased neuron levels (41%) (30). Furthermore, due to the young age the mice experienced tremors and Purkinje cell density loss suggests that Pur α is required for neuronal development. Apart from Pur α -mediated FXTAS pathogenesis, a proteomic approach of transgenic FXTAS fly model(rCGG₉₀) identified Pur α co-localization with Rm62, a Pur α -interacting protein that mediates rCGG-toxicity (31). Rm62 is involved in *HSP70* mRNA exportation. Furthermore, the transgenic flies(rCGG₆₀) displayed a significant decrease in Rm62 protein levels vs. WT flies. Interestingly, a comparison of total RNA from nuclear and cytoplasmic fractions of transgenic and WT flies revealed transgenic nuclear fractions express a two-fold *HSP70* transcript increase along with differentially expressed stress genes such as HSPs and attacin (31). These findings suggest Pur α -mediated Rm62 sequestration into *FMR1*mRNA aggregates impairs Rm62-mediated mRNA transport. The increase in stress-response transcripts reflects an initial response to the CGG-stress; however, impairment of Rm62-mediated nuclear exportation interferes with the neuron's ability to produce proteins that respond to cellular stress. These studies display how Pur α , and Pur α -interacting proteins become impaired in FXTAS.

FMRpolyG/A aggregates have been identified in FXTAS brain samples. Interestingly, these aggregates stained positively for ubiquitin and HSPs (5,14). Recent studies using nuclear magnetic resonance spectroscopy revealed that Pur α binds DNA and RNA the same way (32). Therefore, FMRpolyG/A-induced aggregates formation sequestration of Pur α may also contribute to FXTAS.

Huntington's disease, disruption of axonal transport and neuropathy

HAP1 (huntingtin-associated protein 1) is a protein involved in intracellular trafficking (33); however, HAP1 becomes impaired in HD, resulting in aberrant axonal transport. Previous studies identified the co-localization of HAP1 within mHtt aggregates, while others have shown mHtt-HAP1 co-localization within microtubules and lysosomal organelles (33). KIF5 (kinesin-related motor protein) is a microtubule-associated protein involved in organelle transport. KIF5 and HAP1 mediate GABA_A-receptor (GABA_AR) trafficking. GABA_ARs regulate the strength of inhibitory synaptic plasticity (34). Live cell microscopy analysis of transfected mHtt(Q₁₀₉) neurons revealed a significant decrease in GABA_AR velocity, clustering and decreased inhibitory post-synaptic currents (IPSC); these are similar findings to purposely inhibited HAP1-KIF5

complex formation, suggestive of mHtt-mediated impaired GABA_AR localization. A separate study also identified decreased GABA_AR IPSC in a transgenic mouse model(mHtt-Q₈₂). Interestingly, aberrant IPSCs were observed early during in symptomatic HD model (3–5 months old) and not in presymptomatic mice (35). The symptomatic HD mice developed loss of coordination and gait, which suggests that impaired KIF5-dependent GABA_AR trafficking might contribute to the altered information processing seen in middle-late stage HD. However, another study displayed that presymptomatic 2-month-old mice (Q₈₂) did not experience altered IPSCs (35). It is interesting to consider whether aberrant GABA_AR trafficking might play a role in the epileptic seizures experienced by children with early HD. Furthermore, we cannot exclude the possibility of additional KIFs-HAP1 interactions and their influence on GABA_AR trafficking. Disrupted GABA_AR trafficking to synapses is likely to result in an increased excitotoxic synaptic response, contributing HD pathogenesis. This hypothesis is likely, given a polyQHtt-mediated NMDAR excitotoxicity has been proposed in HD.

Huntington's disease, disruption of axonal transport and neuropathy

Normal huntingtin induces the transcription of the brain-derived neurotrophic factor (BDNF), a neuroprotective factor required for MSNs development (36). TrkB is the receptor for BDNF. mHtt and impaired BDNF might explain the neuronal loss and motor alterations seen in HD. Striatal sample analyses from young R6/2 HD mice models displayed normal full-length-TrkB and BDNF expression but significant neuronal degeneration; however, active-phosphorylated-TrkB levels were decreased vs WT mice (36). Furthermore, R6/2 striatal culture exposed to high BDNF-doses displayed minor TrkB and Erk1/2 signaling increase (36); this finding reveals that mHtt impairs BDNF-induced TrkB and Erk1/2 pathway activation. These findings suggest that active TrkB levels are decreased in young HD mice prior any obvious neuronal morphology changes. Downstream defects in TrkB signaling pathways might underlie early MSN deficits seen in HD and result in BDNF reductions and neuronal loss as seen in late-stage HD. Although conflicting reports describe normal BDNF levels in HD brain samples (37), reduced neurotrophic support might not rely only on BDNF. Interestingly, the ERK1/2 pathway promotes cell death by suppressing survival pathways; therefore, attenuated ERK1/2 activity might be representative of an abnormal autophagy in HD with possible links to neuronal death

(38). Although the exact mechanism by which mHtt interacts with TrkB is unknown, it is known that TrkB is mediated by CREB. CREB binds to a CREB-TrkB promoter site to induce its transcription (39). Downregulation of CREB has been reported in HD (39). Therefore, mHtt impairs CREB-activity, which contributes to aberrant TrkB-BDNF signaling, resulting neurotrophic support loss and neuronal death. Other proteins shown to interact with mHtt are shown in Table 1.

Interactor	Function	Consequence of mHtt interaction	Reference
β-Tubulin	Vesicle transport	Disrupts intracellular transport and insulin secretion	53
CBP	Transcription factor	Cognitive deficits Diminished histone3 acetylation	54
HIP1	Regulates apoptosis and gene expression	Transcriptional deregulation Increased apoptosis	55
NFκB	Transcription factor	Results in increase in inflammatory players implicated in disease progression and neurodegeneration	56
Calmodulin	Ca ²⁺ regulator	Increase intracellular [Ca ²⁺] Results in (+) calpain and causes cytoskeletal and perimembrane protein degeneration	57

Table 1. Proteins interacting with huntingtin protein resulting in impaired protein function.

FXTAS, disruption of axonal transport and neuropathy

RNA-binding protein hnRNPA2/B1 is involved in RNA processing and transport (2). Sequestration of hnRNPA2/B1 into IBs might mediate FXTAS pathogenesis. Indeed, insertion of FXTAS patient-derived rCGG₉₀ DNA fragments into *Drosophila* model disrupted eye morphology and induced cell death (40). However, in a separate study, hnRNPA2/B1 overexpression decreased rCGG-mediated eye toxicity, suggesting that hnRNPA2/B1 mediates rCGG-neurodegeneration (41). EMSA analysis revealed that BC1 RNA, an effector of translational control, interacts with hnRNPA2/B1 via a 5'BC1 G-A motif (42). However, introduction of RNA-(CGG₁₀₅) significantly reduced dendritic delivery of BC1, suggestive of competitive rCGG-hnRNPA2/B1 binding. rCGG expansions induce conformational changes that form hairpin structures sharing GA motif features (42), resulting in the sequestration of hnRNPA2/B1 into *FMR1*mRNA aggregates. At normal *FMR1*mRNA repeats, hnRNPA1/B2 interaction is regular. Upon CGG-expansion, hnRNPA2/B1 binding increases in favor of aberrant *FMR1*mRNA levels,

thus impairing BC-dendritic delivery. Furthermore, other hnRNPA2/B1-interacting proteins were found to co-localize in rCGG aggregates, including CUBP1. CUBP1 is an RNA-binding protein involved in mRNA stability (42). These finding suggests that rCGG aggregates can directly and indirectly sequester hnRNPA2/B1 and hnRNPA2/B1-binding proteins, thus impairing the transport of regulatory mRNAs and RNAs and seeding aggregate formation. Interestingly, Muslimov *et al.*, (42) questioned whether CGGs mediate RNA targeting. Indeed, transfection of “healthy” CGG₂₄ into α -tubulin RNA displayed increased dendritic delivery compared with CCC₂₄transfected repeat (42). This finding indicates that CGGs contain spatial codes for dendritic RNA targeting, which is an important finding given polyglycine expansions with associated spatial codes might explain the increased *FMR1*mRNA IBs seen in impaired dendritic signaling. It would be interesting to consider how transient alterations of the localization signals of toxic *FMR1*mRNA might reduce dendrite localization and associated neuronal loss.

FXTAS, disruption of axonal transport and neuropathy

Sam68 is an RNA-binding protein involved in cellular processes required for hippocampal physiology. A previous study identified Sam68 sequestration into mRNA aggregates in transgenic mice(CGG₉₈) (43). Interestingly, these mice experienced motor defects, suggestive of Sam68 involvement in the tremor and ataxia symptoms of FXTAS (43). Neurexin-1 mRNA is another Sam68-substrate (44). Neurexins are synaptic receptors involved in presynaptic terminal assembly (44). Therefore, impaired SAM68 function might also account for neurexin-1-mediated hippocampal synapse loss. Sam68 domain mapping identified an RNA-binding region that mediates sequestration of other RNA-binding proteins. For example, COS-7(rCGG₉₀) cells induced sequestration of MBLN1 and hnRNP-G, while deletion of the domain abolished aggregate co-localization (43). These findings indicate that Sam68 mediates the sequestration of multiple proteins, sequestering them from their normal functions promoting hippocampal degradation. Upon 24h-post rCGG transfection, Sam68 aggregate co-localization occurred quickly (43), which suggests that Sam68 is an early mediator of aggregate formation which induces sequestration of important proteins. For example, early β -actin sequestration

might explain focal muscle weakness while attention deficits could result from impaired neurexin. Other proteins shown to interact with *FMR1*mRNA are shown in Table 2.

Interactor	Function	Consequence of <i>FMR1</i> mRNA interaction	Reference
Drosha/DGCR8	microRNA biogenesis	Decreased gene regulation	2
Caprin-1	RNA-binding protein	Decreased RNA neuronal translation	58
TRA2A/B	mRNA splicing regulator	Splicing defects associated with intellectual disabilities	55
Transferrin	Iron regulator	Decreased O ₂ transport, impaired mitochondrial respiration, impaired CNS signal transduction	60
KHDRBS1	Splicing regulator and mRNA transport	Dysregulation of immune system activity which may result in sustained inflammation and tissue damage and cell death	61
Table 2. Proteins or RNAs interacting with <i>FMR1</i> mRNA transcript.			

Therapeutic tools and future directions

Symptomatic treatment

Currently there are no treatments for HD and FXTAS, but there are medications that can help alleviate symptoms. TBZ is one of the FDA-approved HD drugs for chorea (45) (Table 3). However, patients taking TBZ have experienced adverse reactions including sleep disturbances and hyperkinesia (45). Previous reports describe positive responses for β -blockers and primidone treatment for FXTAS tremor and ataxia (46). However, reports have revealed them to be effective in six out of eleven patients (46). Although these are somewhat effective symptomatic treatments, further research is required to find a cure for these conditions.

Drug	Mechanism of action	Outcome	Reference
Creatine – HD	Facilitates ATP recycling	Improve mitochondria function and decrease muscle wasting Effect varies per patient	62
Deutetrabazine -HD	Believed to reverse monoamines from nerve terminals	Decrease chorea symptoms	63
Laquinimod – HD	Believed to compromise immunomodulatory effects of T-cells	Increase BDNF, decrease apoptosis and decrease associated-tissue inflammation	64
Topiramate - FXTAS	Blocks voltage-dependent Na ⁺ and	Reduce tremor Effect varies per patient	65

	Ca ²⁺ channels. Reduces excitatory glutamate pathway		
Memantine - FXTAS	Non-competitive Aspartate receptor antagonist	Improve motor coordination and memory	66
Allopregnanolone - FXTAS	Positive allosteric modulator of the GABA _A receptor	Improved cognitive and GABA _A metabolism	67
Table 3. Example of some of the therapeutic compounds used to treat HD and FXTAS. Some of the medications appear to be efficient at treating TRDs but effects vary per patient. These drugs simply alleviate some of the TDRs symptoms but are not cures.			

Gene targeting therapy - Reducing protein and mRNA expression

RNAi utilize synthetic modifiers (siRNA) that bind to target regions for degradation. The potential of adeno-associated virus(AAV)-mediated expression of siRNA has been examined in nonhuman-primate putamen samples (47). The results revealed a significant decrease in striatal mHtt mRNA levels and improved motor functions (47). Although this is a promising result in terms of treatment, a major challenge in the clinical implementation of RNAis is their delivery past the blood-brain barrier. This challenge can be overcome via convection enhanced delivery (CED). In CED, RNAis are synthesized under positive pressure to increase their distribution volume rather than relying on passive diffusion (47). Currently, clinical trials are underway for RNAis such as AMT-130. AMT-130-treated transgenic minipig HD models displayed a significant reduction in mHtt aggregates by 68% and 47% in the striatum and cortex, respectively, 6-months post-treatment (48). Gene targeting therapy for FXTAS is still under study via reductions in toxic *FMR1* mRNA and FMRP. However, this method might not be as feasible, given certain FMRP levels are required for normal physiological processes. Therefore, gene targeting therapy that reduces *FMR1*mRNA build-up represents a conflict between decreasing toxic mRNA but maintaining functional FMRP. Other clinical trials are shown in Table 5.

Clinical trials
Dose-response Evaluation of the Cellavita HD Product in Patients with Huntington's Disease (ADORE-DH)
Nilotinib in Huntington's Disease (Tasigna HD)
Resveratrol and Huntington Disease (REVHD)
Within Subject Crossover Study of Cognitive Effects of Neflamapimod in Early-Stage Huntington Disease
Diaphragmatic Breathing and Heart Rate Variability Training for Improving Hypertension in Fragile X Associated Tremor/Ataxia
Brain Network Activation and Gait and Posture in FXTAS (FXTAS-BNA)
Citocoline for Treatment of FXTAS
Table 5. Active clinical trials in HD and FXTAS (ClinicalTrials.gov accessed 3 September 2019).

Many trials are underway in hope to find a cure for TRDs; however, these trials have yet to release their results to the public; thus, drug effect on conditions are unknown.

Future directions

Technological advances have enabled the identification of protein-protein and protein-RNA interactions. Understanding the interactions between molecules can aid in elucidating molecular functions and discovery of drug targets. Protein-Protein-Interaction-Optimizer (PIPINO) is a new program that detects protein-protein interactions. PIPINO facilitates the selection of binding partners and compares interaction network dynamics (49). Kalkhoff *et al.*, (49) support PIPINO over co-immunoprecipitation, as such method precipitate background proteins due to non-specific interactions yielding false-positive results. PIPINO can identify potential protein-protein interactions that contribute to HD pathogenesis. catRAPID is a new program that can identify protein-RNA interactions by estimating their binding propensities (50). For example, catRAPID analysis of rCGG identified multiple RNA-protein interactions including co-localizing (hnRNP-G) and non-colocalizing (FMRP) proteins (50). catRAPID results are in agreement with previous data showing co-localization of hnRNPA2/B1 within *FMR1*mRNA aggregates (42). The agreement between in silico and primary data can identify discrepancies between studies, facilitating the identification of mRNA-protein interactions that contribute to FXTAS.

Future directions

A new, innovative approach includes CRISPR/CAS9 genome editing. In brief, an in vitro assay using ipluripotent stem cells from FXTAS patients was combined with CRISPR/CAS9 in hopes of introducing a DNA double-stranded break and activating repair mechanisms (51). Indeed, a reduction in and removal of CGG repeats were observed and surprisingly, edited neurons displayed reactivation of *FMR1*. CRISPR/CAS9 is an exciting approach to gene correction for HD and FXTAS. However, further research is required to understand the impact of gene reactivation.

Conclusion

HD and FXTAS pathogeneses are mediated by complex interactions throughout the body. Due to the large number of affected intracellular pathways, it is difficult to pinpoint

exactly where the mutation has gone wrong without considering the downstream effects. Our understanding of the pathogenic mechanisms of TRDs has substantially increased in recent years. While many aspects of TRD toxicity remain a mystery, the data analyzed in this review hopes to contribute to our understanding of how protein sequestration impairs the proteostasis network and neurogenesis, thus elucidating how these alterations mediate HD and FXTAS neuropathogenesis. Although no effective therapy for HD and FXTAS has been identified, the aforementioned therapies shed light onto the possibility of finding a cure for these conditions.

References

1. Budworth H, McMurray C. A Brief History of Triplet Repeat Diseases. *Methods Molecular Biology*. 2013;3(17).
2. Rubinsztein D. The Molecular Pathology of Huntington's Disease (HD). *Current Medicinal Chemistry - Immunology, Endocrine and Metabolic Agents*. 2003;3:329–40.
3. Wu LL, Zhou X-F. Huntingtin associated protein 1 and its functions. *Cell Adhesion & Migration*. 2009 Jan;3(1):71–6.
4. Paulson H. Repeat expansion diseases. *Handbook of clinical neurology*. 2018;147:105–23.
5. Glineburg MR, Todd PK, Charlet-Berguerand N, Sellier C. Repeat-associated non-AUG (RAN) translation and other molecular mechanisms in Fragile X Tremor Ataxia Syndrome. *Brain Research*. 2018 Aug;1693(1693):43–54.
6. Hagerman P. Fragile X-associated tremor/ataxia syndrome (FXTAS): pathology and mechanisms. *Acta Neuropathologica*. 2013 Jun 21;126(1):1–19.
7. Xi W, Wang X, Laue TM, Denis CL. Multiple discrete soluble aggregates influence polyglutamine toxicity in a Huntington's disease model system. *Scientific Reports*. 2016 Oct 10;6(1).
8. Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, et al. Huntingtin-Encoded Polyglutamine Expansions Form Amyloid-like Protein Aggregates In Vitro and In Vivo. *Cell*. 1997 Aug;90(3):549–58.
9. McGowan DP, van Roon-Mom W, Holloway H, Bates GP, Mangiarini L, Cooper GJS, et al. Amyloid-like inclusions in Huntington's disease. *Neuroscience*. 2000 Oct;100(4):677–80.
10. Chen M, Wolynes PG. Aggregation landscapes of Huntingtin exon 1 protein fragments and the critical repeat length for the onset of Huntington's disease. *Proceedings of the National Academy of Sciences*. 2017 Apr 11;114(17):4406–11.
11. Zainelli GM, Ross CA, Troncoso JC, Muma NA. Transglutaminase Cross-Links in Intranuclear Inclusions in Huntington Disease. *Journal of Neuropathology & Experimental Neurology*. 2003 Jan;62(1):14–24.

12. Lesort M, Chun W, Tucholski J, Johnson GV. Does tissue transglutaminase play a role in Huntington's disease? *Neurochemistry International*. 2002 Jan;40(1):37–52.
13. Todd PK, Oh SY, Krans A, He F, Sellier C, Frazer M, et al. CGG Repeat-Associated Translation Mediates Neurodegeneration in Fragile X Tremor Ataxia Syndrome. *Neuron*. 2013 May;78(3):440–55.
14. Krans A, Skariah G, Zhang Y, Bayly B, Tod P. Neuropathology of RAN translation proteins in Fragile X-associated Tremor/Ataxia Syndrome. *bioRxiv*. 2019;1–35.
15. Schaffar G, Breuer P, Boteva R, Behrends C, Tzvetkov N, Strippel N, et al. Cellular Toxicity of Polyglutamine Expansion Proteins. *Molecular Cell*. 2004 Jul;15(1):95–105.
16. Li L, Liu H, Dong P, Li D, Legant WR, Grimm JB, et al. Real-time imaging of Huntingtin aggregates diverting target search and gene transcription. *eLife*. 2016 Aug 3;5:e17056.
17. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional Repression of PGC-1 α by Mutant Huntingtin Leads to Mitochondrial Dysfunction and Neurodegeneration. *Cell*. 2006 Oct;127(1):59–69.
18. G. Vonsattel JP, DiFiglia M. Huntington Disease. *Journal of Neuropathology and Experimental Neurology*. 1998 May;57(5):369–84.
19. Hosp F, Gutiérrez-Ángel S, Schaefer MH, Cox J, Meissner F, Hipp MS, et al. Spatiotemporal Proteomic Profiling of Huntington's Disease Inclusions Reveals Widespread Loss of Protein Function. *Cell Reports*. 2017 Nov;21(8):2291–303.
20. Wyttenbach A, Carmichael J, Swartz J, Furlong RA, Narain Y, Rankin J, et al. Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proceedings of the National Academy of Sciences*. 2000 Mar 14;97(6):2898–903.
21. Labbadia J, Cunliffe H, Weiss A, Katsyuba E, Sathasivam K, Seredenina T, et al. Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *Journal of Clinical Investigation*. 2011 Aug 1;121(8):3306–19.
22. Li RL, M K, F Y, L P, D D, T M, et al. Poly-glutamine Expanded Huntingtin Dramatically Alters the Genome Wide Binding of HSF1. *Journal of Huntington's Disease*. 2012;1(1):33–45.

23. Gomez-Pastor R, Burchfiel ET, Neef DW, Jaeger AM, Cabisco E, McKinstry SU, et al. Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. *Nature Communications*. 2017 Feb 13;8(1).
24. Miranda DR, Wong M, Romer SH, McKee C, Garza-Vasquez G, Medina AC, et al. Progressive Cl⁻ channel defects reveal disrupted skeletal muscle maturation in R6/2 Huntington's mice. *The Journal of General Physiology*. 2016 Nov 29;149(1):55–74.
25. Iwahashi CK, Yasui DH, An H-J, Greco CM, Tassone F, Nannan K, et al. Protein composition of the intranuclear inclusions of FXTAS. *Brain*. 2005 Oct 24;129(1):256–71.
26. Arocena DG, Iwahashi CK, Won N, Beilina A, Ludwig AL, Tassone F, et al. Induction of inclusion formation and disruption of lamin A/C structure by premutation CGG-repeat RNA in human cultured neural cells. *Human Molecular Genetics*. 2005 Oct 20;14(23):3661–71.
27. Garcia-Arocena D, Yang JE, Brouwer JR, Tassone F, Iwahashi C, Berry-Kravis EM, et al. Fibroblast phenotype in male carriers of *FMR1* premutation alleles. *Human Molecular Genetics*. 2009 Nov 11;19(2):299–312.
28. van de Klundert FAJM, Gijzen MLJ, van den IJssel PRLA, Snoeckx LHEH, de Jong WW. α B-crystallin and hsp25 in neonatal cardiac cells — differences in cellular localization under stress conditions. *European Journal of Cell Biology*. 1998 Jan;75(1):38–45.
29. Khalili K, Del Valle L, Muralidharan V, Gault WJ, Darbinian N, Otte J, et al. Pur Is Essential for Postnatal Brain Development and Developmentally Coupled Cellular Proliferation As Revealed by Genetic Inactivation in the Mouse. *Molecular and Cellular Biology*. 2003 Sep 12;23(19):6857–75.
30. Barbe MF, Krueger JJ, Loomis R, Otte J, Gordon J. Memory deficits, gait ataxia and neuronal loss in the hippocampus and cerebellum in mice that are heterozygous for Pur-alpha. *Neuroscience*. 2016 Nov;337:177–90.
31. Qurashi A, Li W, Zhou J-Y, Peng J, Jin P. Nuclear Accumulation of Stress Response mRNAs Contributes to the Neurodegeneration Caused by Fragile X Premutation rCGG Repeats. Orr HT, editor. *PLoS Genetics*. 2011 Jun 2;7(6):e1002102.

32. Weber J, Bao H, Hartlmüller C, Wang Z, Windhager A, Janowski R, et al. Structural basis of nucleic-acid recognition and double-strand unwinding by the essential neuronal protein Pur-alpha. *eLife*. 2016 Jan 8;8(5):e11297.
33. Wu LL, Zhou X-F. Huntingtin associated protein 1 and its functions. *Cell Adhesion & Migration*. 2009 Jan;3(1):71–6.
34. Twelvetrees AE, Yuen EY, Arancibia-Carcamo IL, MacAskill AF, Rostaing P, Lumb MJ, et al. Delivery of GABAARs to Synapses Is Mediated by HAP1-KIF5 and Disrupted by Mutant Huntingtin. *Neuron*. 2010 Jan;65(1):53–65.
35. Yuen EY, Wei J, Zhong P, Yan Z. Disrupted GABAAR trafficking and synaptic inhibition in a mouse model of Huntington's disease. *Neurobiology of Disease*. 2012 May;46(2):497–502.
36. Nguyen KQ, Rymar VV, Sadikot AF. Impaired TrkB Signaling Underlies Reduced BDNF-Mediated Trophic Support of Striatal Neurons in the R6/2 Mouse Model of Huntington's Disease. *Frontiers in Cellular Neuroscience*. 2016 Mar 9;10(37).
37. Ferrer I, Goutan E, Marín C, Rey M., Ribalta T. Brain-derived neurotrophic factor in Huntington disease. *Brain Research*. 2000 Jun;866(1–2):257–61.
38. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, et al. Autophagy and apoptosis dysfunction in neurodegenerative disorders. *Progress in Neurobiology*. 2014 Jan;112:24–49.
39. Deogracias R, Espliguero G, Iglesias T, Rodríguez-Peña A. Expression of the neurotrophin receptor trkB is regulated by the cAMP/CREB pathway in neurons. *Molecular and Cellular Neuroscience*. 2004 Jul;26(3):470–80.
40. Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, et al. RNA-Mediated Neurodegeneration Caused by the Fragile X Premutation rCGG Repeats in *Drosophila*. *Neuron*. 2003 Aug;39(5):739–47.
41. Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, et al. RNA-Binding Proteins hnRNP A2/B1 and CUGBP1 Suppress Fragile X CGG Premutation Repeat-Induced Neurodegeneration in a *Drosophila* Model of FXTAS. *Neuron*. 2007 Aug;55(4):565–71.

42. Muslimov IA, Patel MV, Rose A, Tiedge H. Spatial code recognition in neuronal RNA targeting: Role of RNA–hnRNP A2 interactions. *The Journal of Cell Biology*. 2011 Aug 1;194(3):441–57.
43. Sellier C, Rau F, Liu Y, Tassone F, Hukema RK, Gattoni R, et al. Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. *The EMBO Journal*. 2010 Feb 25;29(7):1248–61.
44. Iijima T, Wu K, Witte H, Hanno-Iijima Y, Glatter T, Richard S, et al. SAM68 Regulates Neuronal Activity-Dependent Alternative Splicing of Neurexin-1. *Cell*. 2011 Dec;147(7):1601–14.
45. Frank S. Tetrabenazine as anti-chorea therapy in Huntington Disease: an open-label continuation study. Huntington Study Group/TETRA-HD Investigators. *BMC Neurology*. 2009 Dec;9(1).
46. Neurology Today. FXTAS: A Syndrome still missed - How and When to Detect and Test for It [Internet]. Neurology Today. American Academy of Neurology; 2019 [cited 2019 Sep 5]. Available from:
https://journals.lww.com/neurotodayonline/Fulltext/2005/08000/Fxtas__A_Syndrome_Still_Missed__How_and_When_To.1.aspx?trendmd_shared=0
47. Stiles DK, Zhang Z, Ge P, Nelson B, Grondin R, Ai Y, et al. Widespread suppression of huntingtin with convection-enhanced delivery of siRNA. *Experimental Neurology*. 2012 Jan;233(1):463–71.
48. Valls A, Brouwers C, Pintauro R, Snapper J, Bohuslavova B, Sogorb-Gonzalez M, et al. I05 Sustained mutant huntingtin lowering in the brain and cerebrospinal fluid of huntington disease minipigs mediated by AAV5-MIHTT gene therapy. *Journal of Neurology, Neurosurgery & Psychiatry*. 2018;89:A89–90.
49. Kalkhof S, Schildbach S, Blumert C, Horn F, von Bergen M, Labudde D. PIPINO: A Software Package to Facilitate the Identification of Protein-Protein Interactions from Affinity Purification Mass Spectrometry Data. *BioMed Research International*. 2016;2891918:1–13.
50. Cirillo D, Agostini F, Klus P, Marchese D, Rodriguez S, Bolognesi B, et al. Neurodegenerative diseases: Quantitative predictions of protein-RNA interactions. *RNA*. 2012 Dec 21;19(2):129–40.

51. Park C-Y, Halevy T, Lee DR, Sung JJ, Lee JS, Yanuka O, et al. Reversion of FMR1 Methylation and Silencing by Editing the Triplet Repeats in Fragile X iPSC-Derived Neurons. *Cell Reports*. 2015 Oct;13(2):234–41.
52. Buijsen R. Fragile X-associated Tremor/Ataxia Syndrome: RNA or RAN? Erasmus University [Internet]. 2016;16. Available from: <https://pdfs.semanticscholar.org/d409/4f0cf4bad6e150e8320b6067634feccdf2b9.pdf>
53. Smith R, Bacos K, Fedele V, Soulet D, Walz HA, Obermuller S, et al. Mutant huntingtin interacts with α -tubulin and disrupts vesicular transport and insulin secretion. *Human Molecular Genetics*. 2009 Jul 23;18(20):3942–54.
54. Giralt A, Puigdemívol M, Carreton O, Paoletti P, Valero J, Parra-Damas A, et al. Long-term memory deficits in Huntington's disease are associated with reduced CBP histone acetylase activity. *Human Molecular Genetics*. 2011 Nov 24;21(6):1203–16.
55. Bhattacharyya NP, Banerjee M, Majumder P. Huntington's disease: roles of huntingtin-interacting protein 1 (HIP-1) and its molecular partner HIPPI in the regulation of apoptosis and transcription. *FEBS Journal*. 2008 Jul 15;275(17):4271–9.
56. Labadorf A, Hoss AG, Myers RH. Huntington's Disease. *Neuroimmune Pharmacology*. 2016 Dec 23;503–16.
57. Kolobkova YA, Vigont VA, Shalygin AV, Kaznacheyeva EV. Huntington's Disease: Calcium Dyshomeostasis and Pathology Models. *Acta Naturae*. 2017 Jun 1;9(2):34–46.
58. El Fatimy R, Tremblay S, Dury AY, Solomon S, De Koninck P, Schrader JW, et al. Correction: Fragile Mental Retardation Protein Interacts with the RNA-Binding Protein Caprin1 in Neuronal RiboNucleoProtein Complexes. Brosius J, editor. *PLoS ONE*. 2012 Sep 27;7(9).
59. Cid-Samper F, Gelabert-Baldrich M, Lang B, Lorenzo-Gotor N, Ponti RD, Severijnen L-AWFM, et al. An Integrative Study of Protein-RNA Condensates Identifies Scaffolding RNAs and Reveals Players in Fragile X-Associated Tremor/Ataxia Syndrome. *Cell Reports* [Internet]. 2018 Dec [cited 2019 Sep 5];25(12):3422-3434.e7. Available from: <https://www.sciencedirect.com/science/article/pii/S2211124718318679>
60. Ariza J, Steward C, Rueckert F, Widdison M, Coffman R, Afjei A, et al. Dysregulated iron metabolism in the choroid plexus in fragile X-associated tremor/ataxia syndrome. *Brain*

Research [Internet]. 2015 Feb [cited 2019 Sep 5];1598:88–96. Available from:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4340768/>

61.Jalnapurkar I, Rafika N, Tassone F, Hagerman R. Immune mediated disorders in women with a fragile X expansion and FXTAS. *American Journal of Medical Genetics Part A*. 2014 Nov 14;167(1):190–7.

62.Hersch SM, Schifitto G, Oakes D, Bredlau A-L, Meyers CM, Nahin R, et al. The CREST-E study of creatine for Huntington disease. *Neurology*. 2017 Jul 12;89(6):594–601.

63.Dean M, Sung V. Review of deutetrabenazine: a novel treatment for chorea associated with Huntington’s disease. *Drug Design, Development and Therapy*. 2018 Feb;Volume 12:313–9.

64.Ralf Reilmann, Mark Forrest Gordon, Anderson KE, Feigin A, Tabrizi SJ, Leavitt BR, et al. The Efficacy and Safety Results of Laquinimod as a Treatment for Huntington Disease (LEGATO-HD) (S16.007). *Neurology [Internet]*. 2019 Apr 9 [cited 2019 Sep 5];92(15 Supplement):S16.007.

Available from: https://n.neurology.org/content/92/15_Supplement/S16.007

65.Berry-Kravis E, Hall D, Leehey M, Hagerman H. Treatment and Management of FXTAS. In: *The Fragile X-Associated Tremor Ataxia Syndrome (FXTAS)*. New York: Springer, New York, NY; 2010. p. 1–5.

66.Ortigas MC, Bourgeois JA, Schneider A, Olichney J, Nguyen DV, Cogswell JB, et al. Improving Fragile X-Associated Tremor/Ataxia Syndrome Symptoms With Memantine and Venlafaxine. *Journal of Clinical Psychopharmacology*. 2010 Oct;30(5):642–4.

67. Napoli E, Schneider A, Wang JY, Trivedi A, Carrillo NR, Tassone F, *et al*. Allopregnanolone Treatment Improves Plasma Metabolomic Profile Associated with GABA Metabolism in Fragile X-Associated Tremor/Ataxia Syndrome: a Pilot Study. *Molecular Neurobiology*. 2018 Sep 5;56(5):3702–13.

68. Hannan AJ. Tandem repeats mediating genetic plasticity in health and disease. *Nature Reviews Genetics*. 2018 Feb 5;19(5):286–98.