Review:

DNA repair in trinucleotide repeat ataxias

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Abstract

The inherited cerebellar ataxias comprise of a genetic heterogeneous group of disorders. Pathogenic expansions of cytosine-adenine-guainine (CAG) encoding polyglutamine tracts account for the largest proportion of autosomal dominant cerebellar ataxias, while GAA expansion in the first introns of *frataxin* gene is the commonest cause of autosomal recessive cerebellar ataxias. Currently, there is no available treatment to alter the disease trajectory, with devastating consequences for affected individuals. Inter and Intra family phenotypic variability suggests the existence of genetic modifiers, which may become targets amendable to treatment. Recent studies have demonstrated the importance of DNA repair pathways in modifying spinocerebellar ataxia with CAG repeat expansions. In this review, we discuss the mechanisms in which DNA repair pathways, epigenetics and other cis-acting factors may act as modifiers in cerebellar ataxias due to trinucleotide repeat expansions.

Keywords: Ataxia, Genetic Modifiers, Genetic association, DNA repair, polyglutamine

Introduction

Over the last decades, scientists have identified 3,300 causative genes of the ~4,900 Mendelian disorders in human (1, 2). The identification of genetic variants informed us the pathogenesis of the inherited ataxias. However, there is still substantial deficiency in our understanding of the factors that modify disease severity. For instance, affected individuals with the same length of genomic CAG repeat expansion in *ATXN3* can have markedly divergent disease course (3). The varying disease manifestations of single-gene human diseases may depend on their interactions with various cis- and trans-acting genetic factors. These genetic modifiers are postulated to exert their influence by altering the disease penetrance, expressivity, dominance and pleiotrophy (4). They may target the mutant genes or gene products, augment the pathophysiological process or provide functional compensation through alternative pathways. We are only beginning to dissect the complex mechanisms of genetic modifiers through delineation of molecular pathways in the era of high-throughput sequencing. Genome wide association study, transcriptome wide association study and other protein network analyses are important approaches to identify these novel pathways (5-7). Genetic Modifiers of Huntington's Disease Consortium is one such example (8). Better understanding of the genetic modifiers will also provide us with insights into novel targets that may be tractable to treatments. In this review, we aim to summarise the DNA repair mechanisms and genes that are good candidates for the search of genetic modifiers in trinucleotide repeat ataxias.

Autosomal Dominant Cerebellar Ataxia

Spinocerebellar ataxias (SCA) are autosomal dominantly inherited neurodegenerative disorders that predominantly affect the cerebellum and brainstem (9). The core symptoms

include gait ataxia, clumsiness and dysarthria. Patients may also develop pyramidal, extrapyramidal signs, ophthalmoplegia and cognitive impairment in specific SCAs. There are now up to 37 pathogenic genes identified (10). Expansions in coding CAG (a form of trinucleotide repeat (TNR)) encoding for polyglutamine tracts account for 45% of the total population of autosomal dominant inherited ataxias. These include SCA1 (*ATXN1*)(11), SCA2 (*ATXN2*)(12), SCA3 (*ATXN3*)(13), SCA6 (*CACNA1A*)(14), SCA7 (*ATXN7*)(15), SCA17 (*TBP*)(16) and Dentatorubral pallidoluysian atrophy *(ATN1)*(17). Despite of the diverse number of pathogenic genes, the underlying pathogenic mechanism is proposed to involve the toxic polyglutamine protein (18). In contrast, the hallmarks of non-coding repeat expansions in SCAs are transcribed nuclear accumulation of repeat RNA-binding proteins that can cause RNA toxicity and lead to disease pathogenesis.(19) The 'conventional' mutations (eg missense and nonsense) and the non-coding expansions are only responsible for 8% of all SCA population (20). However, the remainder of autosomal dominant inherited ataxias do not yet have a known pathogenic mutation.

Expansions of trinucleotide repeat (TNR) tracts in dividing cells during germ cell development are responsible for the phenomenon of anticipation whereas expansions in non-dividing somatic cells are postulated to be responsible for phenotypic variation (21). The length of TNR expansion above the disease threshold is inversely proportional to the age of onset (22, 23). This relationship varies between diseases and the difference in age at onset is not accounted for solely by the TNR repeat length. A substantial portion of this residual variance is heritable (3, 24).

Autosomal Recessive Cerebellar Ataxia

Autosomal recessive (AR) ataxias are a genetic heterogeneous group of complex inherited neurodegenerative disorders that usually manifest in infancy/ childhood. The most common

autosomal recessive ataxia is Friedreich's ataxia (FRDA). FRDA is a (GAAGAAGAA)n repeat expansion disorder in intron 1 of the *frataxin* gene on the positive strand of chromosome 9q21.11 leading to epigenetic changes within the *frataxin* gene, culminating in heterochromatin-mediated transcriptional silencing of the gene(25). FRDA is characterized by cerebellar and sensory ataxia, with involvement of pyramidal tracts (26). Patients may also suffer from cardiomyopathy, optic neuropathy, skeletal deformity and diabetes mellitus. As in SCA, age at onset and progression of FRDA partly depends on the TNR repeat length (27). However, genetic modifiers may also account for the somatic instability of the expanded (GAA)n that drives disease progression (28).

The identification of Genetic Modifiers

The clinical diversity of the hereditary cerebellar ataxias in terms of age of onset, progression and severity of disease, strongly suggests the presence of modifying factors. The investigation of genetic loci that impact variation is required to elucidate the biological mechanisms underlying disease manifestation in these disorders. Insights into these underlying pathways could potentially serve as a target for drug manipulation. A recent review by Holmans et al. provides an excellent summary in the identification of genetic modifiers (5). It has been suggested that common genetic variants, with a significant effect size, may act as genetic modifiers in rare Mendelian conditions such as the SCAs. Genome Wide Association Studies (GWAS) are an effective way to identify such common variants (29), but achieving sufficient power for rare conditions is challenging. Effective collaboration has successfully identified modifiers of age of onset in Huntington's disease, also a trinucleotide repeat disorder (5). In brief, this identified three genome-wide significant loci with enrichment in DNA repair networks. Subsequently, genotyping of these SNPs in

patients with CAG repeat disease (consisting of 1017 SCA patients), showed a significant association between DNA repair genes and the age at onset of SCA and Huntington's disease, with SNPs in FAN1, PMS2 and RRM2B reaching the lowest p-values(30). These findings suggest that common DNA repair pathways act on trinucleotide repeat expansion modifying expression (31, 32).

The role of DNA repair in the hereditary ataxias

Effective DNA repair is fundamental to the development of the nervous system. It is essential during neurogenesis, particularly the proliferative expansion phase (33). Similarly, deficient DNA repair in the mature brain is associated with aging and neurodegenerative conditions such as Alzheimer's Disease (34). Neurons have a substantial requirement for oxidative metabolism, which produces free radicals, and can result in single strand break (SSB) in DNA. These breaks can potentially impact gene expression through interference in transcription and also result in apoptosis (35, 36). Purkinje cells appear particularly vulnerable to oxidative stress (37). The association between DNA repair and the hereditary ataxias was established with the discovery that Ataxia Telangiectasia occurs due to mutations in the *ATM* gene, leading to double strand breaks (DSB), apoptosis and neurodegeneration (38). Since then, ineffective DNA repair has been implicated in several hereditary ataxias. Mutations in genes involved in the repair of DNA double strand breaks, such as Nijmegen breakage and Seckel Syndrome, result in the most severe phenotypes with extraneural involvement. The severity of these conditions is likely attributable to inhibition of the repair process due to the lack of an unbroken complementary DNA strand to use as a template. Such breaks can therefore result in disruption of chromosomal structure. Additionally, mutations in genes that modulate single-strand repair can impair RNA polymerase advancement through

transcription and have been implicated in a number of hereditary ataxias. Notably, Ataxia-ocular motor apraxia 1 (39), Spinocerebellar ataxia with axonal neuropathy 1 (40), and Ataxia-ocular motor apraxia 4 (41), occur due to mutations in *APTX*, *TDP1* and *PNKP*, respectively. These genes synthesize end-processing enzymes pivotal to single strand break repair, and also play an important role in the repair of double strand break by the non-homologous end joining pathway (31, 42, 43). Similarly, in trinucleotide repeat disorders such as SCAs, DNA repair regulation is an essential process to maintain integrity of expansions in replication and translation (21, 44).

Maintenance of genomic integrity requires intricate interplay between multiple repair pathways, cell cycle checkpoints, signal-transduction and effector systems connected with replication, transcription and differentiation (45). The key steps of DNA repair pathways are detection of DNA modification, recruitment of repair factors, removal or modification of the damaged DNA, processing of DNA ends, resynthesis of the missing nucleotides and ligation of DNA strands. The major DNA repair mechanisms include base excision repair, mismatch repair, nucleotide excision repair, double-strand DNA break repair and interstrand cross-link repair (46). Figure 1 outlines the different mechanisms which DNA repair pathways influence the expansion repeat tracts and they are discussed in details below.

Mechanisms of DNA repair

Cross Link Repair

The integrity of FAN1 is speculated to play a role in preventing TNR expansion (30). FAN1 is a repair nuclease that is recruited to sites of crosslink damage. It was initially thought to act through the Fanconi anemia pathway with FANCI-FAND2 complex depending on FAN1's

ubiquitin binding domain (UBZ) (47). More recently, a FAN-1 deficient mouse model demonstrates that it has cross-link repair activities that are independent of the Fanconi anemia proteins (48). FAN-1 prevents replication fork collapse and controls their progression binding with ubiquitylated proliferating cell nuclear antigen (PCNA) accumulated at stalled forks (49). When polymerase encounters a trinucleotide repeat (TNR) tract on the leading strand template, replication fork stalls within repeated DNAs (50). The replication fork regresses to form a four-way junction on the lagging strand in order to bypass the TNR track block (51). A loop intermediate within the TNR tract is formed as a hairpin on the daughter strand of the lagging strand template (52, 53). Fork stalling result in chromosomal fragility and increased vulnerability to interstrand crosslink formation and may lead to TNR expansion.

Mismatch Repair

The GeM-HD GWA study reveals genes that mismatch repair (MMR) proteins are significantly associated with variation in age of onset of patients with Huntington Disease (HD) (54). There is substantial biological evidence that links MMR with trinucleotide repeat disorders. Crossing of HD CAG knock-in mice onto backgrounds deficient in MMR genes show that Msh3 is required for somatic instability with CAG expansion and early disease phenotype in striatum whilst Msh6 protect against intergenerational contractions (55). MMR pathway consists of at least six MutS and MutL homologues (56). MutSα recognizes single base mismatches whilst MutSβ recognizes small insertion/ deletion loops. MutS heterodimers then recruit MutLα and MutLβ, which are essential for subsequent excision and resynthesis. MutLα is the second heterodimer consisting of MLH1 and PMS2, which are endonucleases. TNR tracts are susceptible to strand-slippage and hairpin formation or extrahelical loop extrusion during DNA replication, repair, or recombination (57). MutSβ-provoked strand

excision is followed by gap resynthesis. If the strand breaks generated by MutLα are in close proximity, excision on both DNA strands would lead to a double-strand break. Repair of the double-strand break could then lead to expansion of TNR tracts. Therefore, both MMR proteins that directly bind mismatched DNA and the proteins subsequently recruited to the complex (PMS2 and MSH3) play an important role in TNR tract expansions(58-60). This is further supported by high expression of MSH3 in human postmitotic neurons and MSH3 protein levels correlate with the degree of instability of an expanded CAG repeat transgene (61). However, mismatch repair system protects against intergenerational GAA repeat instability in Friedreich ataxia mouse model (62). This implies that MMR may play a different role in germline TNR tracts.

Base excision repair

Base excision repair (BER) pathway is intimately linked to the MMR pathway. BER handles simple modifications such as alkylations and oxidations of single bases. Several ataxic disorders are characterised by BER gene mutations. These include oculomotor apraxia type 1 and 4 with mutations in aprataxin (APTX) and polynucleotide kinase 3'-phosphatase (PNKP) respectively. CAG repeats are particular vulnerable to oxidative DNA damage, resulting in 7,8 dihydro-8-oxoguanine (8-oxoG) lesions (21). The 8-oxoG DNA glycosylase (OGG1) generates an abasic site. Apurinic/apyrimidinic endonuclease1 (APE1) moves to the abasic site and creates strand breaks. Various end-processing enzymes, such as APTX and PNKP can modify the DNA ends. In order to restore the correct Watson-Crick base and ligation to reconnect the broken ends, DNA polymerase β fills the gap by incorporating up to 15 nucleotides in the repair patch (63). However, CAG repeat facilitates strand-displacement DNA synthesis at the strand breaks. Repeated rounds of oxidation-repair-expansion at TNRs result in hairpin loop formation. The hairpin loop also prevents FEN1-mediated cleavage in vitro (64). The unrectified extrahelical loop would then be incorporated into the primary

structure of DNA. In vivo, inactivation of the OGG1 gene suppresses age-dependent expansions of these repeats in transgenic mice with expanded CAG repeat at the Huntingtin gene (65). This suggests that crosstalk of different DNA repair pathways modulate TNR tract length (66).

Nucleotide excision repair

Nucleotide excision repair (NER) pathway targets helix-distorting lesions in DNA. In an association study of 137 parent-child transmissions in Machado-Joseph disease, a variant ERCC6 (Cockayne syndrome protein CSB) is associated with an expansion bias of (CAG)n (67). NER can be divided into transcription-coupled repair and global genomic repair. Transcription-coupled repair recognizes DNA damage at DNA-damage site when transcription is stalled. In global genomic repair, different enzymes such as xeroderma pigmentosum complementation group C (XPC) detect DNA damage on scanning of the whole genome. Mutations in ERCC6 or ERCC8 lead to an autosomal recessive neurodegenerative disorder characterized by progressive cachexia, growth retardation and leukoencephalopathy, named as the Cockayne syndrome (68). Interestingly, a recent case series describes four unrelated patients with slowly progressive cerebellar ataxia, cognitive decline and chorea and they were found to possess *ERCC4* mutations (69). Loss of ERCC6 is specific to transcription-coupled repair and results in contraction of CAG repeats (70). Homozygous nonsense variant in *ERCC5*, a structure specific exonuclease required for making 3' excision during transcription-coupled repair, was reported to be associated with spastic ataxia with severe neuropathy in two siblings (71). In a drosophila model for SCA3, transcription-coupled repair and adenosine 3',5'-monophosphate response element-binding protein, a regulator of DNA repair, modulate CAG-repeat expansions (72). In addition, the

NER scaffold protein Xpa responsible for recruiting and positioning excision nucleases is also implicated in the regulation of TNR tracts in somatic tissues. In a mouse model of SCA1, Xpa deficiency dramatically reduces CAG repeat instability in neuronal tissue but has no effect on germline tissues (73). This suggests that deficient NER may result in somatic expansions in non-expanding cells of repeat expansion SCAs and subsequent disease progression.

Single Strand DNA Repair

Given that neuronal DNA damages are predominantly single stranded, various key regulator genes in single strand DNA break (SSB) may impact on the phenotypic outcomes of hereditary ataxia (74). SSB are discontinuities in one strand of the DNA double helix and are usually accompanied by loss of a single nucleotide and by damaged 5'- and/or 3' termini at the site of the break. The sources of SSBs are oxidative attack by endogenous reactive oxygen species due to BER and erroneous activity of cellular enzymes DNA topoisomerase 1 (TOP1). Various specific DNA end-processing enzymes restore the damaged termini to conventional 3'-hydrocyl and 5'-phosphate moieties prior to gap filling and DNA ligation. Four key proteins of SSB repair pathways, APTX, PNKP, TDP1 and XRCC1, have been implicated in neurodegeneration and autosomal recessive ataxia. Mutated *APTX* gene leads to ataxia-oculomotor apraxia type 1 with features of cerebellar ataxia, axonal neuropathy, cognitive impairment, hypercholesterolaemia, hypoalbuminemia and hyperkinetic movement disorders. APTX produces aprataxin which is a polypeptide of 342 residues attached to a mitochondrial targeting sequence owning to alternative splicing contains a central histidine triad domain (HIT) and is responsible for processing 3'-phosphate and 3'-phosphoglycolate termini and removal of AMP from 5'-terminus of DNA breaks, especially in mitochondria

(75-77). Thus APTX is both an end-processing factor and a DNA deadenylase that can proofread the DNA ligase reaction (78, 79). Similarly, mutation of a gene encoding 3' phosphate and 5'-hydroxyl termini DNA end processing factor, *PNKP* (polynucleotide kinase 3'-phosphatase), results in apraxia with oculomotor apraxia type 4 (80, 81). Mutated of *TDP1* gene results in spinocerebellar ataxia with axonal neuropathy, which is characterized by cerebellar ataxia, peripheral neuropathy, hypercholesterolaemia and hypoalbuminaemia (40). TDP1 is a 3' end-processing factor that repairs TOP1-SSBs (82, 83). Recently, cerebellar ataxia due to mutated *XRCC1* gene was reported in three unrelated patients (84, 85). XRCC1 is a scaffold protein that directly interacts with PNKP, APTX and Polβ and indirectly through DNA ligase IIIα with TDP1 (42). XRCC1 accelerates the overall process of SSB repair, which it does by stabilizing and stimulating its protein partners at sites of chromosomal DNA damage (86, 87). Mutation of XRCC1 leads to SSB sensor protein PARP1 (ribosylated poly-ADP-ribose polymerase 1) hyperactivation and excessive synthesis of poly(ADP-ribose) (84). In a Xrcc1 deficient mouse model, persistence of DNA strand breaks lead to drastic loss of cerebellum interneurons (88). The central role of XRCC1 in SSB repair and availability of PARP1 inhibitor make PARP1 a promising novel target for treating ataxia with repeat expansion tracts (89).

Double Strand DNA Repair

Recent literature has suggested that double strand break (DSB) may be responsible for large scale expansion in non-dividing cells providing a potential mechanism for modification of repeat length and phenotypic variability (90, 91). DSB repair have two major repair pathways: homologous recombination repair (HRR) and nonhomologous end joining (NHEJ) (92). Homologous recombination uses a sister chromatid as a template to repair a DSB

whereas NHEJ ligates together the two DNA ends with little or no processing (43, 46) Whilst mature postmitotic neurones in G0/G1 rely upon error prone NHEJ, break induced replication (BIR) of homologous recombination can cause large scale expansion in a CAG tract repeat cellular model in vitro (31, 91). The TNR expansion occurs during fork stalling resulting from hairpin formation on the single stranded DNA template. BIR is then initiated when a one-ended DSB undergoes 5' strand resection followed by invasion into a homologous sequence. This newly invaded strand containing the repetitive tract then serves as a primer for DNA synthesis using DNA polymerase delta. Importantly, BIR may also occur in non-dividing neurons and drive disease progression (91). A number of autosomal recessive ataxias are driven by genetic mutations in DSB repair genes involved in homologous recombination. Senataxin (*SETX*) mutation causes ataxia with oculomotor apraxia type 2 (93). Senataxin is essential during crossing-over in homologous recombination and disrupted *SETX* gene results in persistence of DNA double-strand breaks and R-loop formation (94). Mutations in *ATM* (ataxia-telangiectasia mutated) and *MRE11* (meiotic recombination 11) result in ataxia-telangiectasia (AT) and AT-like disorders (95, 96). They produce neurodegeneration, especially of the cerebellum, and a predisposition to hematologic cancers and immunodeficiency. ATM is one of the initiating kinase of the checkpoint signalling pathways that commit cells to HRR (97). ATM triggers complex phosphorylation of cell-cycle checkpoint effectors to activate, amplify or silence kinase signalling (98). ATM can also signal to the apoptosis machinery via Chk2 and p53 to activate proapoptotic gene expression and apoptosis (45, 99). MRE11 is involved in MRE11-RAD50-NBS1 (Nijmegen breakage syndrome 1) complex, known as the MRN complex. MRN is a damage sensor complex common to both NHEJ and HRR and determines which pathway is chosen, and is required for normal ATM activation (100, 101). In vitro, DNA damage and neuronal cell death is triggered by chronic activation of ATM signalling pathway in SCA3 due to

inactivation of PNKP (94, 102). For further detailed discussion of ATM signalling in DSB repair, excellent reviews are available on this topic (33, 103, 104).

Epigenetic factors

Epigenetics exerts control over gene expression and phenotypic manifestations without altering an individual's DNA sequence. It controls development, tissue differentiation and cellular responsiveness. The main three forms of epigenetics are DNA methylation, post-translational modifications of nucleosomal histones and higher-order chromatin structure (105). The drivers of epigenetic changes are yet to be fully elucidated. In adult somatic cells, methylation is primarily found on CpG nucleotides. Environmental factors such as diet, deficiency in essential amino acids and folate, and exposure to nicotine and other toxins have been linked to changes in global methylation changes (106-108). The mechanisms that dictate DNA heterochromatinisation (dense packaging of DNA leading to transcriptional silence) may be associated with dose dependent effects of heterochromatin modifiers and several transcription factors (109, 110). Figure 2 outlines some of the epigenetic factors that influence phenotypic manifestation of repeat expansion ataxias and they are discussed in details below.

Recently, elevated global DNA methylation was observed in SCA 1 and 2 (111). In a SCA7 mouse model, CTCF (CCCTC binding factor) acts as a trans-acting factor that interacts in a methylation-dependent manner with the adjacent cis-environment to prevent expansion of CAG repeat (112). CTCF is an evolutionarily conserved zinc-finger DNA binding protein with activity in chromatin insulation, transcriptional regulation and genomic imprinting (113, 114). It mediates long-range chromatin interactions, directing DNA segments into transcription factories. CTCF is also essential for cohesion localization (115), and insulate

promoters from distant enhancers and controls transcription at insulin-like growth factor 2 locus (116, 117).

In 2400 individuals with trinucleotide repeat disorders, flanking genomic DNA GC content correlates with expansion of CAG repeat tract (118). These flanking DNA regions lie within CpG islands, which are sites of transcription initiation; and they are also methylation targets for DNA methyltransferase (119). In addition, the CpG islands destabilize nucleosomes and attract proteins that create a transcriptionally permissive chromatin state (120). The authors hypothesized that the flanking DNA may modify the downstream processing of aberrant structures by DNA repair machinery through either R-loops or CpG methylation effect and/or chromatin dynamics such as CTCF (112, 121, 122). Indeed, higher methylation levels in CpG island in SCA3 patients have earlier age at onset with the same size of CAG repeats (123). In SCA2 patients, CpG methylation in *ATXN2* gene is associated with CAG expansions (124). However, complete knockdown of DNA methyltransferase in human and mouse cell lines results in CAG repeat instability (125). Loss of *DNMT1* function also causes a form of autosomal dominant cerebellar ataxia with deafness and narcolepsy (126). Together, this suggests that the stability of TNR tract requires our cells to maintain a normal methylation pattern and DNMT1 is essential to mediate appropriate transcriptional repression.

In FRDA, epigenetic changes play a significant role in modifying Frataxin gene expression. Abnormal (GAA)n expansion forms heterochromatin, which mediates variegated silencing (127). Hypoacetylation of histones H3 and H4 and trimethylation of histone H3 at Lys9 induce epigenetic promotor silencing and impair transcriptional initiation and elongation (128-130). This promotor silencing is related to repeat length (131). Histone deacetylase inhibitors reverse the silencing in vitro (132). Further trials with histone deacetylase inhibitor Nicotinamide in FRDA patients to explore its long term clinical efficacy are warranted (133).

Additional Genetic Modifying Elements

In the EuroSCA cohort of 1255 individuals with SCA type 1, 2, 3, 6 and 7, age at onset of SCA2, 3, 6 and 7 are influenced by other (CAG)n-containing genes (134). Expanded alleles of SCA 1, 6 and 7 also interacts with normal alleles in trans. This was partially replicated in independent population and separate studies in different ethnic groups (135-138), though not evident in SCA2 individuals from South America (139). Mitochondrial polymorphism A10398G correlates with earlier age of onset and cognitive decline phenotype in SCA2 (140, 141); whereas glucocerebrosidase gene variants are associated with parkinsonism phenotype in SCA3 (142). Apolipoprotein E (ApoE) e2 allele reduces the age of onset by 4-5 years compared to others who carry ApoE e3/e3 or e3/e4 in 192 SCA3 patients (143). Biological basis of these associations remain uncertain.

Other cis-acting elements such as repeat interruptions and variants in 3' untranslated regions also regulate repeat associated instability. Interruptions of a pure CAG repeat reduces stability of hairpin structures formed by pure (CAG)n repeat in DNA (144). This inhibits repeat expansions and inserts a number of histidines into the polyglutamine stretch of proteins (145). This interruption in SCA2 may even produce different phenotypes such as parkinsonism or Amyotrophic lateral sclerosis (146, 147). In a group of 170 Chinese patients with SCA3, carriers of either rs709930 A allele and rs910369 T allele in the 3' untranslated region of ATXN3 result in earlier age of onset between 2-4 years (148). However, no similar modifying single nucleotide polymorphism was found in the 5' regulatory regions of ATXN3 (149). This variant may not be a genetic modifier itself but rather be associated with yet to be discovered casual genetic variants.

Conclusion

We have outlined the recent approaches in the search of genetic modifiers of trinucleotide repeat ataxias. An International GWAS of repeat expansion ataxia would be worthwhile and this is likely to provide further insights into the genetic factors influencing the clinical characteristics of these disorders. As outlined, evidence suggests that genes in DNA repair pathways may play a modifying role. These include genes involved in crosslink, mismatch, base excision, nucleotide excision, single strand and double strand DNA repair. The interplay of these genes is postulated to influence the penetrance, age of symptom onset and progression of ataxia with abnormal repeat expansions. This stimulates strong interest in the importance of DNA repair not only in genetic ataxia disorders with repeat expansions, but also its role in other neurodegenerative disorders. We have also discussed epigenetic factors, interactions of expanded and normal alleles in trans and several other genetic polymorphism and their potential modulating effects on age of onset of repeat expansion ataxias. In future, disentangling the contribution of various DNA repair genes and epigenetic factors to genetic ataxia and other neurodegenerative disorders will provide important insights to potential therapeutic targets for these conditions.

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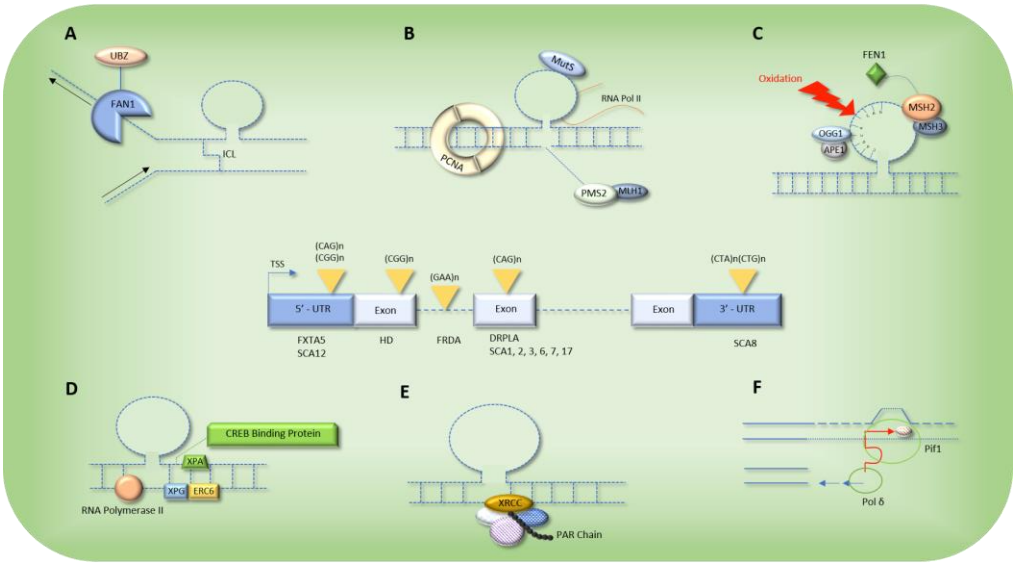
Contributors

WY/ EO: design, draft and revision of manuscript. RS: graph design and revision of manuscript. LA: drafting and revision of manuscript. NWW: critical revision of the manuscript for important intellectual content.



Figures:

Fig 1. Alterations in DNA repair pathway influence the expansion repeat tracts through different mechanisms. (a) FAN1 nuclease is recruited to ICL via UBZ. FAN1 mutation may lead to TNR expansion due to unresolved ICL. The small loop represents hairpin structure formed by TNR. (b) PCNA latches onto the extrahelical extrusion formed by strand slippage. MutS heterodimers then detects the extrusion and recruits MutLα (PMS2 and MLH1). The subsequent double strand break may lead to TNR expansion. (c) Single base excision protein OGG1 generates a basic site after oxidative damage whilst APE1 creates the strand breaks. Repeat rounds of oxidation-repair-expansion results in hairpin formation. The hairpin loop

prevents FEN1 mediated cleavage and escapes further repair by forming complexes with MutSβ (MSH2, MSH3). (d) Transcription-coupled repair recognizes the DNA damages at the TNR tract. Mutations of signaling protein XPA and effector protein ERCC6 result in changes of CAG repeat length. (e) XRCC1 is a scaffold protein that moves to single strand DNA breaks to recruit repair enzymes. Mutations of XRCC1 lead to PARP1 hyperactivation and may contribute to TNR length variation. (f) After one ended double strand break, break induced repair occurs with strand invasion containing TNR tracts, and is mediated by Polδ. It occurs in a migration bubble aided by Pif1 helicase through repetitive tract and continues until it reaches an oncoming replication fork. (CREB- cAMP response element binding protein, DRPLA- dentatorubral-pallidoluysian atrophy, ERCC6- excision repair 6, FAN1- FANCD2/ FANC1-associated nuclease, FRDA- Friedreich's ataxia, FXTAS- fragile X tremor ataxia syndrome, HD- Huntington disease, MLH1- mutL homolog1, MSH2- MutL homolog2, MutL homolog 3, mutS- mutator S, OGG1- oxoguanine glycosylase 1, PCNA- proliferating cell nuclear antigen, PMS2- PMS1-homologue 2, PAR- poly(ADP-ribose), Pif1- PIF1 5' to 3' DNA helicase, Polδ- polymerase delta, RNA Pol II- ribonucleic acid polymerase 2, SCA- spinocerebellar ataxia, TSS- transcription start site, UBZ- ubiquitin binding domain, UTR- untranslated region, XPA- xeroderma pigmentosum complementation group A protein, XPG- xeroderma pigmentosum complementation group G protein, XRCC1 X-ray cross-complementing protein 1)

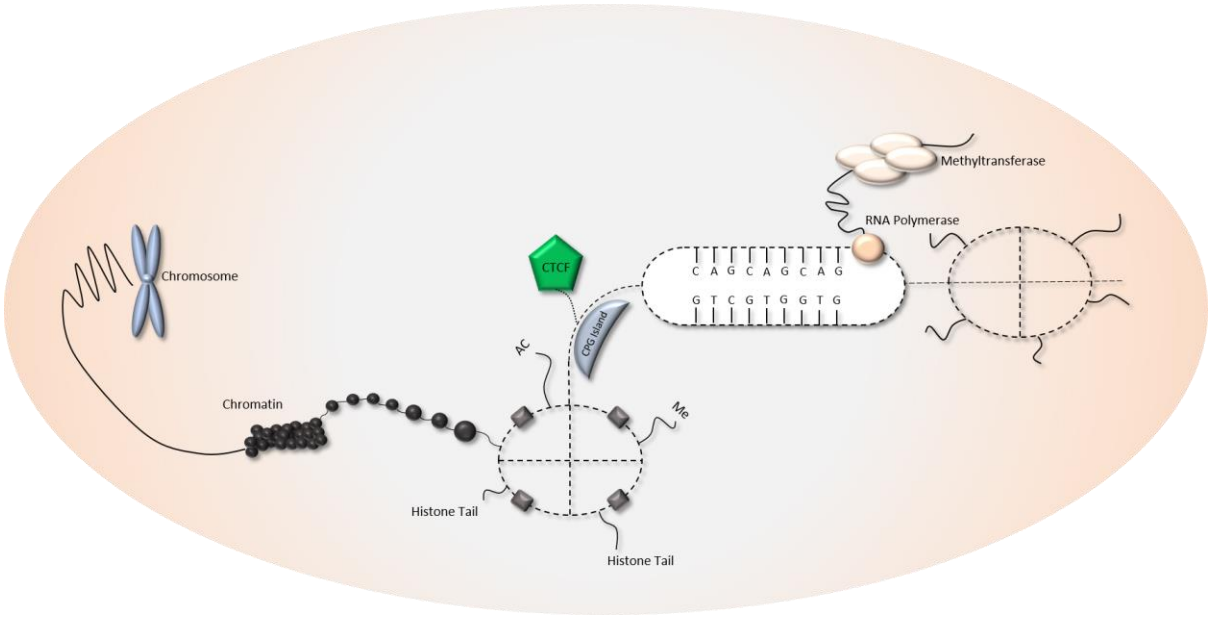


Fig 2. CTCF, CpG island methylation, methyltransferase activity and acetylation and methylation of specific histones are some of the epigenetic factors that influence phenotypic manifestation of hereditary ataxia with repeat expansions. They act via accessibility of DNA through altering chromatin structure, transcriptional repression and promotor silencing. (AC-acetylation, CTCF- CCCTC binding factor, ME-methylation)

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