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Review article

Fragile X premutation carriers: A systematic review of neuroimaging findings

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ABSTRACT

Background: Expansion of the CGG repeat region of the *FMR1* gene from less than 45 repeats to between 55 and 200 repeats is known as the fragile X premutation. Carriers of the fragile X premutation may develop a neurodegenerative disease called fragile X-associated tremor/ataxia syndrome (FXTAS). Recent evidence suggests that premutation carriers experience other psychiatric difficulties throughout their lifespan.
Methods: Medline, EMBASE and PsychINFO were searched for all appropriate English language studies published between January 1990 and December 2013. 419 potentially relevant articles were identified and screened. 19 articles were included in the analysis.
Results: We discuss key structural magnetic resonance imaging (MRI) findings such as the MCP sign and white matter atrophy. Additionally, we discuss how functional MRI results have progressed our knowledge of how FXTAS may manifest, including reduced brain activation during social and memory tasks in multiple regions.
Limitations: This systematic review may have been limited by the search for articles on just 3 scientific databases. Differing techniques and methods of analyses between research groups and primary research articles may have caused differences in results between studies.
Conclusion: Current MRI studies into the fragile X premutation have been important in the diagnosis of FXTAS and identifying potential pathophysiological mechanisms. Associations with blood based measures have also demonstrated that neurodevelopmental and neurodegenerative aspects of the fragile X premutation could be functionally and pathologically separate. Larger longitudinal studies will be required to investigate these conclusions.

Contents

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1. Introduction

The fragile X-associated tremor/ataxia syndrome (FXTAS) is one of the most prevalent movement disorders with a known single gene causation [1]. FXTAS is a neurodegenerative disease which affects approximately 40% of males and 8–16% of females who carry the premutation allele of the *FMR1* gene [2,3]. At present, there is no evidence based treatment for FXTAS, although symptomatic treatments of associated cognitive, psychiatric and movement disorders have proven useful in a percentage of cases [4].

Premutation status is conferred by an expansion of the non-translated 5′ CGG repeat region of *FMR1* from the normal range, which is less than 45 repeats, to between 55 and 200 repeats. Typically, an expansion of over 200 repeats is associated with DNA methylation and subsequent silencing of *FMR1* leading to a lack of production of a protein called fragile X mental retardation protein (FMRP). This lack of FMRP manifests clinically as the severe neurodevelopmental disorder fragile X syndrome [1]. The premutation allele is unstable and the CGG repeat region is liable to expand through maternal transmission. Thus, a mother with the fragile X premutation is very likely to have a child with fragile X syndrome [5].

1.1. Clinical features associated with the fragile X premutation

The classical clinical presentation of FXTAS is late-onset, usually male and over 50 years of age, with progressive symptoms of tremor, ataxia and cognitive decline. Gait ataxia, kinetic tremor and mild Parkinsonism typically are the first symptoms to appear in FXTAS [6]. Patients begin to experience frequent falls, and eventually become bed bound in the later stages of the disease. Peripheral neuropathy, dysfunction of the autonomic system and endocrine changes also form part of the FXTAS phenotype, however these occur less frequently [7]. Onset of cognitive decline is initially subtle and typically precedes appearance of motor symptoms. Cognitive decline in FXTAS mainly involves deficits in executive function, working memory, inhibition and visuospatial learning and progresses to full dementia in approximately 50% of patients [8,9]. In patients with established FXTAS gross changes to white matter structure can be seen in almost all individuals using magnetic resonance imaging, suggesting that disturbances to brain connectivity underpin the disorder [10]. It is of note that FXTAS symptomatology is both broad and heterogeneous, with similarities to multiple other diseases, likely resulting in under- and misdiagnoses. Psychiatric problems (including, anxiety, irritability and obsessive–compulsive behaviours) and autistic traits have been identified in premutation carriers throughout their lifespan [11]. Such traits are also known to be associated with disturbances to executive function and changes to brain connectivity.

1.2. Molecular changes associated with the fragile X premutation

Unlike in fragile X syndrome, where the expansion exceeds 200 CGG repeats, the premutation allele remains unmethylated, and as such encodes a functional transcript of FMRP. FMRP is expressed at highest concentrations in the brain and is a transcriptional regulator with a diversity of functions. Most importantly it is heavily involved in the regulation of synaptic maturation and plasticity [1,12]. In carriers of the premutation, production of *FMR1* mRNA increases up to 8-fold the normal level, likely due to changes in expansion size altering chromatin structure and giving increased access to transcriptional modulators of the FMRP gene [13]. In addition, FMRP levels have been observed to be slightly lower in some individuals with the premutation, especially at the high end of the CGG repeat range [13–15]. The causation for this is debated, but it has been suggested that a fall in FMRP could arise from deficits in the mRNA translational efficiency [13]. It is possible that this small decrease in FMRP may contribute to increased rates of neurodevelopmental abnormalities in premutation carriers, including autistic behaviours. However, it is widely accepted that the high level of *FMR1* mRNA in premutation carriers is the major causative factor in the molecular pathology of FXTAS [16]. Indeed, studies have shown that intranuclear inclusions in neurones and astrocytes, which are a pathological hallmark of FXTAS, are still formed without the FMRP coding region of the gene, and do not form without the CGG repeat expansion [17]. It seems that the mRNA has a toxic gain-of-function effect, which proceeds to disrupt numerous cellular pathways to cause neuronal damage or death. In particular, intranuclear inclusions containing *FMR1* mRNA are present throughout the brain and brainstem. The exact mechanism of their formation is not fully understood, however the favourable theory is that an excess of *FMR1* mRNA begins to sequester mRNA binding proteins such as histones, heat shock proteins and cytoskeletal proteins. In particular, neurofilament isoforms lamin A/C have been shown to often be involved in inclusion formation, which is likely to initiate neurofilament dysregulation and may be a major cause of peripheral neuropathy in FXTAS patients. These intranuclear inclusions likely not only cause physical blockages to cellular functions, but have knock-on effects through the sequestering and therefore inhibition of mRNA binding proteins [1,18]. Repeat Associated Non-AUG initiated (RAN) translation has also been implicated in the pathogenesis of FXTAS. The CGG repeat region of the *FMR1* gene has been shown to trigger translation of the polyglycine-containing protein FMRpolyG, despite being outside of the open reading frame. This protein has been demonstrated to be toxic in human cell lines, and to accumulate in intranuclear inclusions in cell culture, mouse models and human FXTAS patients. Given that intranuclear inclusions in FXTAS are ubiquitin-positive, it seems likely that the FMRpolyG protein may significantly contribute to neurodegeneration and it is suggested that in FXTAS, RNA and protein toxicity be additive or synergistic. Similar cases of RAN translation have also been implicated in multiple neurodegenerative diseases, such as ALS and frontotemporal dementia [19]. The antisense transcript AS*FMR1*, which overlaps the CGG repeat region of the *FMR1* gene and is transcribed in an antisense orientation, has also been suggested to contribute to phenotypic variations associated with *FMR1* gene repeat expansions. In a similar way to *FMR1* expression, AS*FMR1* mRNA is upregulated by the premutation allele and silenced by the full mutation. In the premutation, the gene is also alternatively spliced, which also indicates its possible association with FXTAS [20]. Despite the exact mechanisms of *FMR1* mRNA gain-of-function toxicity, pathogenic RAN translation and antisense transcripts being unclear, it is probable that combined down-stream effects cause oxidative stress in neurones and consequent cell damage and apoptosis. Fig. 1 summarises the processes by which the *FMR1* premutation may lead to the clinical features with which it is associated.

Several studies have examined whether CCG repeat length and FMRP levels correlate with the physiological, physical and psychiatric manifestations of the fragile X premutation. It has been identified that in patients with FXTAS, increased CGG repeat sizes are seen to correlate with increased severity of FXTAS symptoms [21]. This has prognostic value as identification of larger CGG repeat size may serve as a risk factor for a more severe form of FXTAS. The relationship between FMRP levels and *FMR1* mRNA levels or the CGG repeat expansion remains unclear, although it is recognised that the *FMR1* protein is modestly reduced

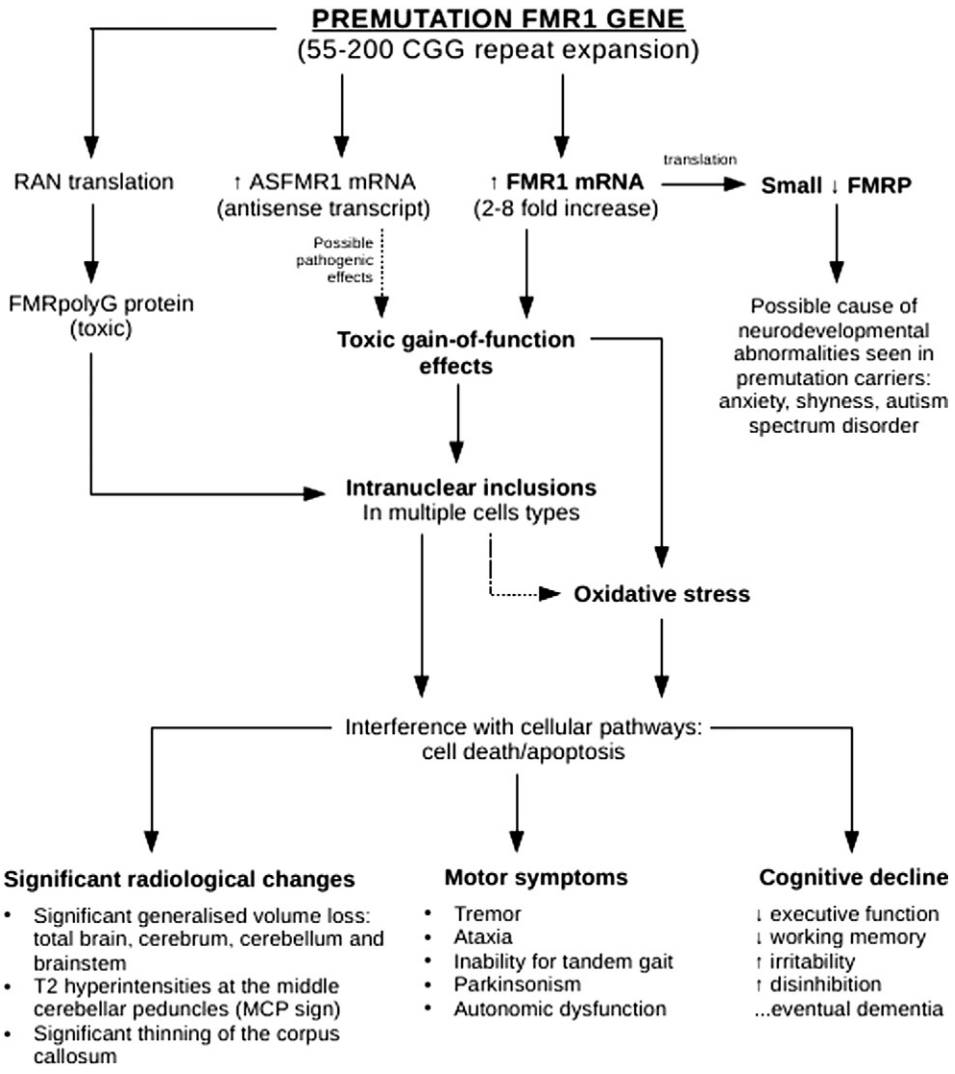

Fig. 1. An overview of the molecular pathology of FXTAS.

by the premutation [13]. This association infers that premutation carriers could also suffer from more neurodevelopmental deficits throughout their life-span due to lower levels of FMRP [22]. In terms of motor symptoms, studies have shown that age of onset, and severity of tremor, ataxia and parkinsonism are positively correlated with CGG repeat size. However, some apparently contradictory results indicate that *FMR1* mRNA levels and symptom severity in FXTAS showed no relationship [6,21]. Severity of neuropathies and speed of nerve conduction scores have also been found to show a positive correlation with CGG repeat size [23]. Test scores into cognition have been shown to correlate with CGG repeat size, with processing speed, executive functioning and perceptual organisation scores decreasing at larger CGG repeat sizes [24,25]. Again, these correlations concerning cognition indicate that individuals who carry larger CGG repeat sizes are at a greater prognostic risk for cognitive decline and eventual dementia. Regarding psychiatric symptomatology, genotype/phenotype relationships are less clear, however higher levels of *FMR1* mRNA have been found in some cases to correlate with increased levels of obsessive–compulsive behaviours, depression, anxiety, hostility and psychoticism [26]. More research is required, however given the important role of FMRP in neurodevelopment, one would expect that FMRP levels may be negatively correlated with psychiatric symptoms.

1.3. Importance of neuroimaging in premutation carriers

Neuroimaging has been a cornerstone in the advancement of our insight into FXTAS and premutation status. Structural and functional MRI techniques have allowed researchers to pinpoint diagnostic criteria and begin to unravel the complex pathology of FXTAS. For example, the high incidence of increased T2 signal intensity at the middle cerebellar peduncles, known as the MCP sign, has become an integral part of the diagnosis for FXTAS [1]. It is hoped that current and future imaging research into the fragile X premutation and FXTAS will reveal more sophisticated measurements of subtle alterations in the brain and help clinicians move towards a more prognostic diagnosis. Here, we systematically review the literature concerning magnetic resonance imaging and the fragile X premutation, with aims to identify strengths, weaknesses and future directions in the research.

2. Methods

Medline, EMBASE and PsychINFO were searched for all English language studies published between January 1990 and December 2013 that reported imaging data in fragile X premutation carriers.

Search terms included "fragile X", "fragile X premutation", "premutation carriers" and related terms using the AND operator with "magnetic resonance imaging". All abstracts were assessed for inclusion and articles were retrieved in full text where appropriate. Out of the 422 abstracts identified by the search, 385 were excluded on the due to lack of relevance to the fragile X premutation and/or neuroimaging. The remaining 37 articles were then assessed individually in full text according to the inclusion criteria. Primary research articles were considered for inclusion if they were published by an English language peer-reviewed journal, used sample groups of fragile X premutation carriers and compared the group(s) to a group of healthy controls using structural, functional or diffusion tensor MRI. The process of study selection is summarised in Fig. 2.

3. Results

3.1. Conventional structural imaging findings

Structural magnetic resonance imaging studies into premutation carriers both with and without signs of FXTAS have revealed major changes in brain structure and connectivity compared to control populations. Indeed, many of the gross radiological changes that occur in premutation carriers have become integral to the diagnosis of FXTAS.

Here, 12 studies considered conventional structural MRI in premutation carriers (summarised in Table 1). Ten studies utilised quantitative structural MRI and one study utilised qualitative analysis of scans. A radiological feature considered to be a prominent hallmark of FXTAS is increased regions of T2 signal intensity in the middle cerebellar peduncles (MCPs), which is known as the MCP sign. The MCP sign was found to be present in FXTAS cohorts in the 4 studies investigating this region. Cerebellar and cerebral atrophy is also very common in patients with FXTAS, with 9 out of 12 studies (both qualitative and quantitative) identifying generalised volume loss in these areas. Premutation carrier groups both with and without established FXTAS showed significant decreases in total brain, cerebrum, cerebellum and brainstem volumes [27,28]. Mild to moderate loss of cerebral cortical volume was seen in 75% of patients exhibiting signs of FXTAS, and 20% of patients showed severe volume loss [29]. Radiological abnormalities and brain atrophy were less severe and less frequent in participants with milder FXTAS symptomatology [30]. In patients with diagnosed FXTAS, the corpus callosum was also found in a majority of cases (14 out of 16 participants) to be significantly thinned in both qualitative and volumetric measurements [10]. Hippocampal and amygdala volumes have also shown volumetric changes, but these have been less clear and findings have been difficult to replicate between groups [27,28,31].

3.2. Correlations between structural imaging findings, molecular measurements and clinical findings

Many conventional structural MRI studies have investigated correlations between radiological abnormalities and molecular measures such as CCG repeat size, FMRP levels and *FMR1* mRNA levels (Table 1). In most cases, neither FMRP nor *FMR1* mRNA levels were seen to associate with any radiological findings. However, CGG repeat size has been identified as a significant predictor of structural changes in the brain in several studies involving various cohorts of male and female asymptomatic premutation carriers and premutation carriers with established FXTAS. Specifically, increased CGG repeat length has been found to associate with reduced cerebellum and total brain volumes and decreased ventricle size [27,28,32]. In addition, voxel based morphometry studies have found that larger CGG repeat sizes are significantly associated with decreased grey matter density in several brain areas and grey matter density in the dorsomedial frontal regions [33,34].

Neuropsychological and clinical measures have also shown to associate with radiological findings and molecular measures. One study identified a significant association between IQ scores and increased ventricle size, as well as volume loss in premutation carriers in multiple brain regions including the whole brain, cerebrum, cerebellum and hippocampus. In addition, higher CGG repeat sizes were associated with lower IQ

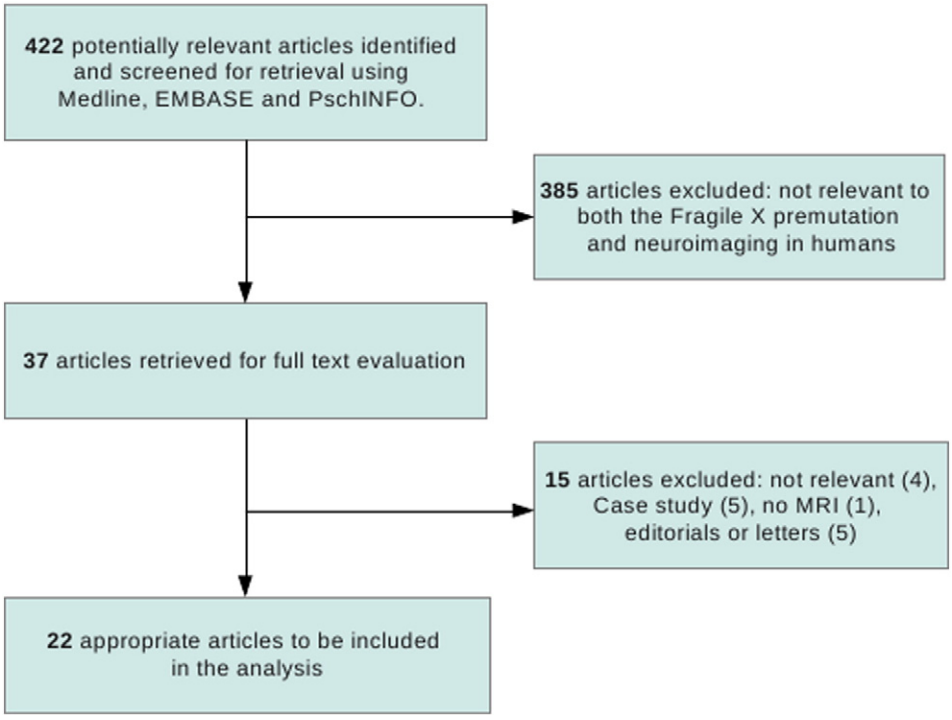

Fig. 2. Inclusion and exclusion process of relevant literature.

scores [28]. Similarly, decreased grey matter in the left inferior frontal cortex and the anterior cingulate cortex was significantly correlated with poor working memory scores and grey matter loss in the left amygdala was significantly associated with higher incidences of obsessive–compulsive and depressive traits [34]. One study also identified that volumetric measurements of the bilateral thalamus and putamen, and left caudate showed significant negative correlation with FXTAS stage [35].

3.3. Diffusion tensor imaging findings

Four relevant structural diffusion tensor imaging (DTI) studies into fragile X premutation carriers were identified in the literature search. DTI has been a useful tool in structural imaging to identify abnormalities in connectivity in premutation carriers. Premutation carriers with FXTAS showed reductions in structural connectivity in motor, limbic, association and callosal fibre tract categories [36]. Specifically regarding motor fibre tracts, premutation carriers with FXTAS had significantly lower tract volume at the descending motor tract, the MCP, the superior cerebellar peduncle and the anterior body of the corpus callosum. Additionally, superior cerebellar peduncle tract volume correlated negatively with CGG repeat length in premutation carriers both with and without FXTAS [37]. Patients with FXTAS showed significant reductions in fractional anisotropy (FA) in numerous white matter tracts, especially the MCPs, the superior cerebellar peduncle, the cerebral peduncle, the fornix and the stria terminalis [38]. Axial and radial diffusivities were also seen to be significantly elevated in the MCPs in premutation carriers with and without FXTAS. In this study CGG repeat size and axial and radial diffusivities at the MCP displayed a U-shaped relationship [38] (Table 2).

3.4. Functional imaging findings

Six papers were identified which utilise fMRI in fragile X premutation carriers. Two considered social tasks, one considered numerical processing and three considered various aspects of memory. In one study, premutation carrier groups both with and without FXTAS generally scored equally as well in working memory tasks performed in the scanner compared to controls. However, the right ventral part of the inferior frontal cortex (vIFC) and the left dorsal part of the inferior frontal cortex and premotor cortex (dIFC/PMC) showed reduced activation in response to the working memory task in both premutation groups [39]. Associative memory recall tasks revealed decreases in left hippocampal activation in asymptomatic premutation carriers. Asymptomatic premutation carriers also displayed an increase in parietal activation compared to controls during this task. During a magnitude estimation task, premutation carriers without FXTAS were additionally found to have significantly lower fronto-parietal activation compared with the control group, specifically in the bilateral inferior parietal lobule and the left inferior frontal gyrus [40]. During emotional processing tasks completed in the scanner, premutation carriers without FXTAS have reliably been shown in two studies to have reduced amygdala activation compared to control groups. Right amygdala activation and overall amygdala activation in particular are decreased [41,42]. Additionally, premutation carriers showed no significant activation in the bilateral superior temporal sulcus, the bilateral orbital gyrus and the bilateral insula during emotional processing tasks, whereas controls showed consistent activation in these areas [42] (Table 3).

3.5. Correlations between functional imaging findings, molecular measurements and clinical findings

Several of the fMRI studies attempted to link imaging findings with clinical and molecular measurements. Functional connectivity analysis has revealed that premutation carriers have a significantly lower connectivity at the right prefrontal cortex and the right parahippocampal gyrus, and this is associated with reductions of FMRP levels up to 23% [14]. Regression analysis also revealed that reductions in FMRP levels were a primary factor in the loss of amygdala activation in premutation carriers [41]. Moreover, decreases in right amygdala volume in one study were closely linked to the presence of psychological symptoms [42]. *FMR1* mRNA levels were also seen to affect brain activation in premutation carriers both with and without FXTAS, as regression analyses indicated a significantly negative influence of increased *FMR1* mRNA on vIFC activity during memory encoding tasks performed in the scanner [39]. Reduced fronto-parietal activation in premutation carriers during a magnitude estimation task was also shown to have molecular correlates, with CGG repeat size displaying a negative relationship with brain activation associated with the distance effect [40].

4. Discussion

4.1. Structural imaging findings

Despite being part of the primary diagnostic criteria for FXTAS, the MCP sign is present in only 60% of FXTAS cases. It is also not exclusive to FXTAS and can be present in multiple other neurological diseases, including multiple system atrophy. Therefore, increased T2 signal in the MCPs indicates that FXTAS is likely, but does not provide a definitive FXTAS diagnosis [43]. In addition, asymptomatic premutation carriers are seen to exhibit milder and less frequent radiological changes, but whether these are predictors of disease is unknown [27]. Much like the MCP sign, thinning of the corpus callosum is thought to be one of the early stereotypical structural changes in the brain preceding the onset of motor symptoms and significant cognitive decline. Interestingly, despite being integral brain areas to FXTAS and premutation carrier symptomatology, hippocampal and amygdala volumes do not seem to be as heavily affected as other brain regions. Hippocampal volumes have been recorded as both significantly increased and not significantly different to control groups [28,44,45]. Similarly, volumetric changes at the amygdala have been noted in premutation carriers, but these findings were not statistically significant [31].

It is as yet unclear whether associations between CGG repeat size and radiological changes correlate with FXTAS severity. While some studies have found a robust correlation [33], others state that molecular measures such as CGG repeat size do not show associations with the severity of FXTAS symptoms [30]. Significant associations were also found between reduced amygdala volume and increased CGG repeat length at the lower range of expansions, but not the higher range [31]. A negative association between CGG repeat size and hippocampal volume was also identified by one study, but this was not significant after adjustment [45]. As illustrated by these often ambiguous correlational findings, the pathology of FXTAS and changes in premutation carriers are heterogeneous and likely occur through many different cellular mechanisms and molecular effectors. Collectively, this is suggestive of CGG repeat size being at least a small factor in determining FXTAS severity, but it is likely that other molecular or cellular changes downstream of repeat size also affect severity of symptoms, resulting in varied findings of associations across groups. This may be reflective of higher CGG repeat sizes causing increasingly larger losses of FMRP production, which could play a part in neurodevelopmental deficits. In terms of psychiatric symptoms, significant negative associations were identified between total hippocampal volume and anxiety in females with and without FXTAS. This association did not prove significant in males, however male premutation carriers exhibited a negative association between hippocampal volume and paranoid ideation [45]. Grey matter loss in the left amygdala is also correlated significantly with increased levels of obsessive–compulsive and depressive traits in males [34]. It is possible that these type of structural changes in the amygdala and hippocampus are driving anxiety-related, depressive and obsessive–compulsive traits, and since such traits are present in carriers both with and without FXTAS, we may expect to see these

Table 1
Conventional structural imaging studies.

Study	Participants	Methodology	Significant findings
Brunberg et al. [10]	17 male PMCs with signs of FXTAS (mean age 68) and 14 male controls (mean age 66 years)	Molecular measures (CGG repeat size, FMRP and *FMR1* mRNA) Conventional structural and volumetric MRI	15/17 PMCs showed symmetrically decreased T1 and increased T2 signal intensities in cerebellar white matter. 14/17 PMCs exhibited the MCP sign. Cerebellar cortical atrophy was present in 16/17 PMCs and cerebral atrophy was present in all PMC participants. The corpus callosum was thinned in 14/16 PMCs and MCPs were atrophic compared to the control group.
Jacquemont et al. [29]	20 male PMCs with FXTAS (aged > 50 years) and 20 matched controls. PMCs recruited through FXS families	Molecular measures (CGG repeat size, FMRP and *FMR1* mRNA) Conventional structural and volumetric MRI	Mild to moderate loss of cerebral cortical volume was present in 75% of patients. The volume loss was severe in 20%. Increased T2 signal intensity in the subependymal and deep white matter of the frontal and parietal lobes was seen in 75% of patients.
Moore et al. [33]	20 male PMCs and 20 male age matched controls. PMCs recruited through FXS families.	Molecular measures (CGG repeat size, FMRP and *FMR1* mRNA) Conventional structural and volumetric MRI	The PMC group had significantly less voxel density in several brain areas including the cerebellum, thalamus and amygdalo-hippocampal complex. Ageing, increased CGG repeat size and decreased FMRP were all associated with decreased voxel density. Regional grey and white matter density is significantly affected in PMCs.
Loesch et al. [44]	24 male PMCs, aged above 33 years and 21 matched controls.	Conventional structural and volumetric MRI	PMCs showed significant decrease in total brain and cerebrum volumes. Volumes of right, left and total hippocampus were significantly increased in PMCs. Significant correlation with decreased brain volume and increasing CGG repeat size
Loesch et al. [27]	12 male PMCs (mean age 62.15) and 11 male matched controls (mean age 62.1). PMCs recruited through FXS families.	Conventional structural and volumetric MRI Cognitive testing Molecular measures (CGG repeat size and *FMR1* mRNA)	Variable MRI changes in PMCs classified as being 'neurologically affected', including cerebral, cerebellar atrophy and the MCP sign
Cohen et al. [28]	11 male PMCs without FXTAS, 25 male PMCs with FXTAS and 21 male matched controls. Aged between 51 and 79 years. PMCs recruited through FXS families.	Conventional structural and volumetric MRI Neurocognitive testing Molecular measures (CGG repeat size and *FMR1* mRNA)	No differences in radiological findings between unaffected PMCs and controls, except for a reduction in brainstem volume Differences in all brain region volumes measured, except for hippocampus, between FXTAS affected and control groups CGG repeat length was associated with the volume of many areas including the cerebellum, ventricle and whole brain white matter hyperintensity. IQ scores were significantly associated with volumes of multiple regions including whole brain, cerebrum, cerebellum, hippocampus, ventricles and whole brain white matter hyperintensity. Higher CGG repeat lengths were correlated with lower IQ scores.
Adams et al. [32]	15 female PMCs with FXTAS (age 59.5 ± 10.3 years), 20 unaffected PMC females (age 43.3 ± 11.2 years), and 11 matched female controls (age 51.0 ± 10.3 years). 36 male PMCs with FXTAS (age 65.0 ± 5.6 years), 25 unaffected PMC males (age 53.5 ± 12.5 years) and 39 matched male controls (age 58.0 ± 15.0 years). PMCs recruited through FXS families.	Conventional structural and volumetric MRI Clinical evaluation Molecular measures (CGG repeat size and *FMR1* mRNA)	Less pronounced reductions of cerebellar volumes and less involvement of the MCP sign was seen in female PMCs compared to male PMCs. Reduced brain volumes and increased white matter disease in PMC females compared to control females Significant associations between reduced cerebellar volume, increased severity of FXTAS symptoms and increased CGG repeat size.
Adams et al. [45]	16 female PMCs with FXTAS (age 57.5 ± 12.46 years), 17 unaffected PMC females (age 44.94 ± 11.23 years), and 8 matched female controls (age 50.63 ± 11.43 years). 34 male PMCs with FXTAS (age 66.44 ± 6.77 years), 21 unaffected PMC males (age 52.38 ± 12.11 years) and 30 matched male controls (age 57.2 ± 14.12 years). PMCs recruited through FXS families.	Conventional structural and volumetric MRI Clinical evaluation Molecular measures (CGG repeat size and *FMR1* mRNA) Psychological symptoms	Significant negative correlation between total hippocampal volume and anxiety in female PMCs with and without FXTAS; this was driven by the significant negative correlation between right hippocampal volume and anxiety. In male PMCs with and without FXTAS, only paranoid ideation negatively correlated with hippocampal volume. Female PMCs also demonstrated a negative association between hippocampal volume and severity of anxiety-related symptoms. Negative association between CGG repeat size and hippocampal volume, but this was not significant after adjustment
Selmeczy et al. [31]	49 PMC males (mean age 48.5 years) and 48 matched controls (mean age 47.9 years). PMCs recruited through FXS families.	Intelligence and psychological testing Molecular measures (CGG repeat size and *FMR1* mRNA) Conventional structural and volumetric MRI	No significant differences between groups in amygdala volumes; Significant negative correlation between amygdala volume and the lower range of CGG repeat expansions, but not the higher range
Juncos et al. [30]	50 male PMCs, with and without FXTAS (mean age 65 years). PMCs recruited through FXS families	Testing for tremor, ataxia and cognitive defects Full neurologic evaluation, including clinical assessments and conventional structural MRI	The majority of PMCs exhibited general volume loss and the MCP sign. CGG repeat size did not seem to correlate with FXTAS severity. Radiological changes were less severe and less frequent in participants with milder FXTAS symptomatology.

Table 1 (*continued*)

Study	Participants	Methodology	Significant findings
Hashimoto et al. [34]	31 male PMCs with FXTAS, 24 male PMCs without FXTAS and 28 male matched controls (aged between 40 and 80 years)	Molecular measures (CGG repeat size and *FMR1* mRNA) Psychological and cognitive assessment Conventional structural and volumetric MRI	Grey matter loss in cortical and subcortical regions was seen in FXTAS patients. Significant associations between grey matter loss in the left amygdala and increased levels of obsessive–compulsive and depressive traits; also significant associations seen between decreased grey matter in the left inferior frontal cortex and anterior cingulate cortex and poor working memory Significant negative effect of CGG repeat size on grey matter density in the dorsomedial frontal regions
Wang et al. [35]	11 male PMCs without FXTAS, 36 male PMCs with FXTAS and 14 male controls (aged between 47 and 81 years)	Conventional structural and volumetric MRI Clinical assessments for presence of FXTAS and psychiatric problems	FXTAS group showed significant atrophy in the bilateral thalamus and putamen, left caudate and right pallidus compared to controls. FXTAS group also showed significant DWI hypointensity in the bilateral thalamus, caudate, putamen and right pallidus compared to controls. Volume measurements of the bilateral thalamus and putamen, and left caudate showed significant negative correlation with FXTAS stage.

Abbreviations.
PMC: premutation carrier; FXS: fragile X syndrome; MCP: middle cerebellar peduncle.

radiological findings throughout the life-span of premutation carriers. Given that molecular measurements in humans are blood-based and not directly sourced from brain tissue, it is currently unclear whether the emergence of these traits is driven by increased levels of *FMR1* mRNA or slightly decreased levels of FMRP, if either. However, one might hypothesise that moderate reductions in FMRP levels could play a role here, given the importance of FMRP for normal neurodevelopment [15]. Further data and investigation into this are necessary to support such conclusions.

4.2. Diffusion tensor imaging findings

Given that the loss of white matter integrity is central to the progressive nature of FXTAS, it is highly likely that deficits in connectivity will play an important role in premutation carriers. Increased axial and radial diffusivities in the MCPs of FXTAS and non-FXTAS premutation carriers are again indicative of changes at the MCPs prior to symptomatic manifestation of FXTAS [38]. In addition, findings suggest that groups with MCP and corpus callosum lesions

Table 2
Diffusion tensor imaging studies.

Study	Participants	Methodology	Significant findings
Hashimoto et al. [38]	35 PMC males with FXTAS, 16 PMC males without FXTAS and 20 matched male controls; participants aged between 40 and 79 years.	Structural MRI: Diffusion tensor imaging Molecular measures (CGG repeat size, FMRP and *FMR1* mRNA)	FXTAS group showed significant reductions in fractional anisotropy (FA) in multiple white matter tracts including the MCPs, superior cerebellar peduncle, cerebral peduncle, fornix and stria terminalis. Axial and radial diffusivities were significantly elevated in the MCP in both premutation groups. U-shaped relationship between CGG repeat size and axial and radial diffusivities in the MCP
Wang et al. [36]	15 PMCs aged under 45 years and 19 matched controls under 45 years (younger groups); 15 PMCs aged over 45 years with FXTAS, 11 PMCs aged over 45 years without FXTAS and 15 matched controls aged over 45 years (older groups). Participants were all male.	Structural MRI: Diffusion tensor imaging	Carriers with FXTAS showed reduced structural connectivity relative to controls in motor, limbic, association and callosal fibre tract categories. Carriers with FXTAS also showed greater age-related decline in structural connectivity in limbic, association and callosal fibre tracts. Carriers with hyperintensities in the MCP and corpus callosum exhibited significantly reduced structural connectivity compared to carriers without hyperintensities.
Battistella et al. [47]	30 PMC males and 37 male matched controls aged between 20 and 70 years. All participants had a family member with FXS.	Global cognitive assessment Neurologic evaluationStructural MRI: Diffusion tensor imaging Molecular measures (CGG repeat size)	Grey matter voxel based morphometry showed a lower grey matter volume in the anterior lobule VI of the cerebellum and bilateral thalamus in PMCs. Radial diffusivity was increased at the MCPs, hippocampal fimbria/fornix and stria terminalis bilaterally. MCP radial diffusivity showed interaction with age and CGG repeat size.
Wang et al. [37]	36 male PMCs with FXTAS, 26 male PMCs without FXTAS and 34 male controls. PMCs were recruited via FXS families	Structural MRI: Diffusion tensor imaging Molecular measures (CGG repeat size and *FMR1* mRNA)	FXTAS group had significantly lower tract volume at the descending motor tract, the MCP, the superior cerebellar peduncle (SCP) and the anterior body of the corpus callosum. Tractography measurements of the corpus callosum and superior cerebellar peduncles showed associations with motor functioning in both premutation groups. CGG repeat length correlated negatively with the SCP tract volume in both PMC groups. CGG repeat length also correlated negatively with SCP FA in the FXTAS group.

Abbreviations: PMC: premutation carrier; MCP: middle cerebellar peduncle.

exhibit significantly reduced structural connectivity as a whole, supporting the notion that the MCPs and the corpus callosum may potentially be important in FXTAS early pathology [36]. DTI studies of premutation carriers are as of yet infrequent, and so this technique could be further utilised in the future to uncover the nature of brain connectivity in both asymptomatic premutation carriers and patients with FXTAS.

4.3. Functional imaging findings

In addition to structural changes in the brain, research has indicated that functional changes and differences in activation are an important consequence of the fragile X premutation. Increased parietal activation in premutation carriers during associative memory recall tasks is suggestive of compensatory activation outside of the hippocampus, given that there was no difference between groups in task accuracy [46]. Compensatory activation may indeed form an important part of the premutation neurological phenotype, and allow premutation carriers to perform normally in tasks and appear asymptomatic during most of their life-span. Additionally, reduced amygdala activation compared to controls during emotional processing tasks may reflect structural findings at the amygdala, such as loss of volume, and also supports findings of significantly increased risk of emotional problems in premutation carriers [1,41]. Premutation carriers also responded to neutral faces presented to them in the scanner with greater overall brain activation than in controls, which may be reflective of psychiatric or neuropsychological symptoms [42]. Findings have, on the whole, been representative of a reduction in the BOLD fMRI signal in premutation carriers with and without FXTAS in multiple areas, however, some studies have reported conflicting results and some groups of premutation carriers have shown no difference in activation amount or patterns during functional testing [14]. This variability of results between studies may arise from differences in tasks used in the scanner or variation in the methods of analyses. It also possible that, given that all but one fMRI study have used asymptomatic premutation carriers, discrepancies may arise due to differences in the likelihood of FXTAS development in the premutation groups.

The use of correlational analyses in fMRI does not infer causation, however they can indicate how changes in functional activation occur, and potentially suggest targets for therapeutic intervention. Links between loss of FMRP and reduction in amygdala activation supports the idea that changes in FMRP levels could be important in the manifestation of the neurodevelopmental symptoms that may exist in premutation carriers [15,41]. Since *FMR1* mRNA is thought to be progressively neurotoxic, the association between increased *FMR1* mRNA and reduced activation at the vIFC during memory encoding suggests

Table 3
Functional imaging studies.

Study	Participants	Methodology	Significant findings
Hessl et al. [42]	12 PMC males (mean age 42.9 years) and 13 male matched controls (mean age 39.8 years)	Psychological assessment (intelligence and psychological symptoms) Molecular genetic measures (CGG repeat size, *FMR1* mRNA) Structural and functional MRI/face processing task Fear potentiated startle and skin conductance paradigms	In PMCs, psychological symptoms were significantly associated with decreased right amygdala volume. PMCs showed an overall decrease in amygdala activation and varied activation patterns. Unlike in controls, PMCs showed no significant activation in the bilateral superior temporal sulcus, bilateral orbital gyrus and bilateral insula. PMCs showed a greater overall activation in response to calm faces.
Koldewyn et al. [46]	11 PMC males (mean age 42.9 years) and 11 matched male controls (mean age 39.8 years).	Psychological assessment (intelligence and psychiatric symptoms) Molecular genetic measures (CGG repeat size, *FMR1* mRNA) Structural and functional MRI/associative memory recall task	Groups did not differ in hippocampal volume. PMCs showed reduced left hippocampal activation and increased right parietal activation during memory recall task compared to controls. Left hippocampal activation was negatively correlated with both *FMR1* mRNA levels and psychiatric symptomatology in the PMC group.
Hashimoto et al. [39]	15 PMCs with FXTAS, 15 PMCs without FXTAS and 12 matched controls. Males and females aged between 33 and 75 years.	Functional MRI and working memory task	All groups performed equally on working memory task. All groups had significant activation in bilateral hippocampus, inferior frontal cortex, premotor cortex, anterior cingulate cortex and supplementary motor area. The right vIFC and left dIFC/PMC showed reduced activation in both PMC groups. Regression analysis showed a significant negative effect of mRNA levels on vIFC activity.
Hessl et al. [41]	23 PMC males (mean age 32.9 years) and 25 matched controls (mean age 30.1 years).	Molecular genetic measures (CGG repeat size, *FMR1* mRNA and FMRP) Structural and functional MRI/emotional processing task	PMCs had significantly smaller right and left amygdala volumes. PMCs had reduced right amygdala activation during emotional processing task. Regression analysis revealed reduced FMRP levels to be a primary factor in the reduced amygdala activation. There was no difference between groups on task accuracy.
Wang et al. [14]	24 PMC males (mean age 32.6 years) and 25 male matched controls (mean age 30.1 years).	Molecular measures (CGG repeat size, *FMR1* mRNA and FMRP) Structural and functional MRI/memory encoding task Psychophysiological interaction analysis	FMRP was 23% reduced in PMCs. No difference in hippocampal/total cerebral volume between groups Both groups had similar performance on encoding task. No significant difference in activation amount or pattern between groups Functional connectivity analysis revealed that PMCs had significantly lower connectivity with the right prefrontal cortex and the right parahippocampal gyrus. This correlated with reduction in FMRP.
Kim et al. [40]	16 female/12 male PMCs (mean age 32.3 years) and 14 female/15 male controls (mean age 30.6 years)	Functional MRI/magnitude estimation task Molecular measures (CGG repeat size and methylation, and *FMR1* mRNA) IQ assessment	The PMC group showed significantly reduced activation in the bilateral inferior parietal lobule and the left inferior frontal gyrus compared to controls for the distance effect in the task. CGG repeat size was a primary factor in reduced fronto-parietal activation in the PMC group.

Abbreviations: PMC: premutation carrier; vIFC: ventral part of the inferior frontal cortex; dIFC/PMC: dorsal part of the inferior frontal cortex and premotor cortex; FXS: fragile X syndrome; MCP: middle cerebellar peduncle.

that deficits in memory and cognition are neurodegenerative in nature, and may be predictive of FXTAS. These results are fundamental in exhibiting the separate nature of the neurodevelopmental and neurodegenerative traits of the fragile X premutation. However, there are to date few fMRI studies of the fragile X premutation and additional larger prospective studies are needed to confirm and extend these findings.

5. Summary

Imaging data in the past two decades has provided us with invaluable information into the nature of the phenotype of the fragile X premutation and FXTAS. Importantly, structural imaging findings have come together to produce a battery of radiological changes that comprise some of the critical diagnostic criteria of FXTAS. Functional imaging have also begun to play a part in this, and have been key in unravelling possible causes of the neurological, psychological and psychiatric changes that underpin much of the premutation phenotype. However, research into FXTAS and the fragile X premutation still requires progression to allow development of targeted treatments in the future. Given the complex nature of the premutation and FXTAS, functional imaging in particular can be further utilised to probe a broad range of clinically relevant features. For example, analysis of emotional processing may help to unravel the neurodevelopmental aspects of the premutation. In addition, longitudinal studies with behavioural, molecular and imaging measures could be useful to identify why FXTAS penetrance is incomplete in premutation carrier samples, which is an important outstanding question in the field. Future studies to discriminate between the proposed neurodevelopmental effects of the CGG repeat expansion and the neurodegenerative aspects of FXTAS are also required. This type of research should subsequently allow a more objective diagnostic criteria for FXTAS, a better understanding of prognosis and contribute towards the development of targeted therapeutics.

Conflict of interest

None.

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