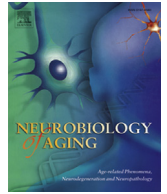




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## Neurobiology of Aging

journal homepage: [www.elsevier.com/locate/neuaging](http://www.elsevier.com/locate/neuaging)Investigation of memory, executive functions, and anatomic correlates in asymptomatic *FMR1* premutation carriers

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## ABSTRACT

Fragile X–associated tremor/ataxia syndrome (FXTAS) is a late-onset movement disorder associated with *FMR1* premutation alleles. Asymptomatic premutation (aPM) carriers have preserved cognitive functions, but they present subtle executive deficits. Current efforts are focusing on the identification of specific cognitive markers that can detect aPM carriers at higher risk of developing FXTAS. This study aims at evaluating verbal memory and executive functions as early markers of disease progression while exploring associated brain structure changes using diffusion tensor imaging. We assessed 30 aPM men and 38 intrafamilial controls. The groups perform similarly in the executive domain except for decreased performance in motor planning in aPM carriers. In the memory domain, aPM carriers present a significant decrease in verbal encoding and retrieval. Retrieval is associated with microstructural changes of the white matter (WM) of the left hippocampal fimbria. Encoding is associated with changes in the WM under the right dorsolateral prefrontal cortex, a region implicated in relational memory encoding. These associations were found in the aPM group only and did not show age-related decline. This may be interpreted as a neurodevelopmental effect of the premutation, and longitudinal studies are required to better understand these mechanisms.

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## 1. Introduction

The *FMR1* premutation is defined by a CGG trinucleotide repeat expansion between 55 and 200 repeats (as opposed to normal alleles <55 CGG) in the 5' untranslated region of the *FMR1* gene (prevalence of 1/100–250 women and 1/250–800 men,

Dombrowski et al., 2002; Rousseau et al., 1995; Toledano-Alhadeef et al., 2001). When transmitted by women, premutation alleles may expand to the full mutation defined by an expansion of more than 200 CGG repeats. The latter causes the fragile X syndrome that is the most frequent form of inherited intellectual disability (prevalence of 1 per 2500, Hagerman, 2008). At the molecular level, the full mutation allele results in transcriptional silencing of the *FMR1* gene and absence of the FMR1 protein (FMRP) (Pieretti et al., 1991), whereas premutation allele results in 2- to 10-fold increased levels of *FMR1* messenger RNA with normal to reduced levels of the FMRP (Allen et al., 2004; Tassone et al., 2000). Carriers of the *FMR1* premutation alleles are at risk of developing fragile X–associated tremor/ataxia syndrome (FXTAS), a late-onset

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neurodegenerative disorder characterized by action tremor, gait ataxia, and parkinsonism and cognitive impairment (Hagerman et al., 2001; Jacquemont et al., 2003; Leehey et al., 2003). The penetrance of FXTAS increases with age, with ~17% of men being affected in their 50s, and 75% of them affected after 80 years (Jacquemont et al., 2004).

Although overall cognitive level of functioning is similar between asymptomatic premutation (aPM) carriers and controls (Allen et al., 2005; Cornish et al., 2011; Grigsby et al., 2008; Hunter et al., 2008), recent studies have shown specific neuropsychological impairments in aPM carriers. Subtle executive deficits affecting working memory and inhibition have been reported in a population of aPM men (Cornish et al., 2009, 2011; Grigsby et al., 2008; Hunter et al., 2012). The central executive component as described in Baddeley model of working memory (Baddeley, 1986) might be particularly vulnerable, as mild executive dysregulation can already be observed in young aPM individuals when the cognitive load on working memory is increased (Cornish et al., 2009, 2011). Hashimoto et al. (2011) using functional magnetic resonance imaging (MRI) found reduced activation of the right ventral and the left inferior frontal cortices and premotor cortex in a verbal working memory task in aPM individuals. This suggests that alterations in the prefrontal cortex may underlie verbal working memory deficits in this population.

In the domain of long-term memory, the reported results in aPM individuals are scarce. Administering the logical memory subtest of the Wechsler Memory Scale (Wechsler, 1997b), Hunter et al. (2008) did not find any differences between aPM carriers and controls on measures of immediate and delayed recalls and delayed recognition. Using the same task, Grigsby et al. (2008) reported deficient performances in immediate and delayed recalls in aPM individuals. Interestingly, tests on a second verbal memory task were normal (Rey Auditory Verbal Learning Test, Spreen and Strauss, 1998). The discrepancies across studies could be attributed to differences in sample composition, number of CGG repeats, and methodological issues. At the imaging level, hippocampal functional alterations have been reported in aPM carriers with memory impairment in a visual task (Koldewyn et al., 2008). The involvement of the hippocampus in patients affected by FXTAS is evident in pathology findings with high rates of intranuclear inclusions further confirmed by evidence for hippocampal atrophy (Greco et al., 2006). Most recent imaging results reported hippocampal fimbria/fornix structure changes in aPM carriers (Battistella et al., 2013).

So far, no studies systematically investigated the relationship between memory impairment and brain structure in aPM carriers. Current efforts are focusing on the identification of markers for accurate detection of aPM individuals at higher risk for developing FXTAS (Cornish et al., 2011; Hunter et al., 2012). It is unknown, however, whether these subtle cognitive deficits and associated structural brain differences represent a slowly degenerative process related to the onset of FXTAS or the neurodevelopmental effect of the premutation. The latter hypothesis has been suggested in studies reporting impairment in cognitive tasks without aging effect in aPM carriers (Cornish et al., 2005, 2009).

The aim of this study was to further investigate specific cognitive alterations in aPM men and their associated brain structural changes. The wide age range of this cross sectional dataset allowed preliminary investigations of the chronology of premutation-related alterations. We focused on the domains of verbal memory and executive functions, in relation to structural alterations previously reported in Battistella et al. (2013). We employed diffusion tensor imaging (DTI), a widely used technique and a sensitive marker of white matter (WM) integrity. Correlation analyses between WM tissue properties and cognitive alterations were performed on a voxel-by-voxel basis. Diffusion-derived maps used for

regression analyses were created using a novel data processing technique that increases tissue specificity and compensates for the effect of the spatial smoothing (Lee et al., 2009).

## 2. Methods

### 2.1. Participants

The study enrolled 30 aPM men ranging from 20 to 70 years of age (median age = 48.2 years, 25th percentile = 39.4, 75th percentile = 58.3). The control group includes 38 men with normal *FMRI* alleles (age range 20 to 66; median age = 43; 25th percentile = 35, 75th percentile = 50). They are spouses of female premutation carriers and are not biologically related to the premutation carriers enrolled in the aPM group. None of the 2 groups (aPM and controls) include multiple members of the same family. The 2 groups were matched for age and educational level. Participants were recruited through fragile X families from Switzerland, France, Italy, and United Kingdom. Brain structure results in these groups of carriers, and controls were recently published (Battistella et al., 2013).

*FMRI* allele status was confirmed in all the participants by DNA testing. Participants were ascertained through a family member with fragile X syndrome. They were included in the aPM group when the CGG repeat size was equal or >55 but <200 and in the control group when the CGG repeat size was <55. Exclusion criteria included (1) the presence of motor symptoms suggestive of possible or probable FXTAS assessed by a movement disorder specialist using 3 standardized movement disorder scales (Fahn et al., 1993; Stebbins and Goetz, 1998; Trouillas et al., 1997); (2) signs of subcortical dementia using the Mattis Dementia Rating Scale (MDRS) with a cutoff of 123 (Mattis, 1976; Sevin et al., 2009); and (3) WM alterations visible on fluid-attenuated inversion recovery (FLAIR) images examined by an experienced neuroradiologist blind to the genetic status of the participants.

Three aPM participants (aged 57, 62, and 66 years) were excluded from the study because of the presence of WM lesions visible on FLAIR images (diffused WM lesions in cerebellum and cerebral hemispheres). They present neither any motor symptom nor signs of subcortical dementia. All participants had normal MDRS scores, and the 2 groups' performances on this scale were not statistically different. The final population then comprised 27 aPM and 38 control subjects. Table 1 lists participants' demographic information, including molecular data and MDRS score.

### 2.2. Neuropsychological assessment

#### 2.2.1. Verbal memory

"Verbal short-term memory" was assessed using the forward digit-span task (Wechsler, 1997a). Participants have to recall increasingly longer sequences of digits presented orally at the rate of 1/second. Two trials are given for each sequence length (9-digit sequence maximum), and task is discontinued after 2 consecutive failures on a given length. The outcome score was the highest number of correctly recalled digit sequences.

"Verbal learning and episodic memory" were assessed with the California Verbal Learning Test (CVLT) (Delis et al., 2000). This task consists of 2 different lists of 16 words (A and B), each comprised 4 words from 4 different categories presented in a pseudorandom manner. List A is repeated 5 times (learning trials) and list B (interference list) once. Four scores were computed on the basis of the CVLT and used as outcome measure: (1) the total number of words correctly recalled across all 5 learning trials (learning score); (2) the number of words recalled after a 20-minute delay (delayed recall); (3) a memory decay score, computed by subtracting the

**Table 1**  
Participant descriptive statistics and *FMR1* CGG repeat size

Descriptive statistics	aPM carriers (n = 27)			Controls (n = 38)			p Value
	Mean	SD	Range	Mean	SD	Range	
Age (y)	46.7	12.5	20–70	42.8	12.3	20–66	.21
CGG repeat size	85.4	26.3	57–156	31.4	6.9	20–54	<.0001
Educational level	2.2	0.8	1–3	2.3	0.7	1–3	.75 <sup>a</sup>
MDRS global score	140.3	3	133–144	141.6	2.6	130–144	.073 <sup>a</sup>

Key: aPM, asymptomatic premutation; MDRS, Mattis Dementia Rating Scale; SD, standard deviation.

<sup>a</sup> Mann-Whitney *U* test.

number of words of the delayed recall to the number of words of the fifth learning trial; and (4) the number of words recognized (yes/no) in a list that encompasses 32 distractors (recognition).

### 2.2.2. Executive function

“Central executive” component of verbal working memory was assessed using the backward digit-span task (Wechsler, 1997a). This task is identical to the forward digit span, but the participants have to recall the digits in the reverse order. The outcome score was the highest number of correctly recalled digit sequences.

“Initiation of verbal response” was assessed through semantic (animal, supermarket) and phonemic (letter M) verbal fluencies in 3 subsequent 60-second sessions. The total number of words for the semantic and phonemic conditions was used as outcome measures.

“Planning of a motor response” was assessed with the Luria 3-step test of motor sequencing. Participants are shown a sequence of 3 hand movements (i.e., fist, side, palm), and they have to realize this sequence 5 times consecutively. A success score was obtained using a binary measure: the participant either succeeded or failed in performing 5 consecutive sequences.

### 2.3. Magnetic resonance imaging

Participants were scanned in a 3-T Siemens Trio scanner (Siemens AG, Erlangen, Germany) using a 32-channel head coil. The protocol included a sagittal T1-weighted gradient-echo (magnetization-prepared rapid gradient-echo) sequence, 160 contiguous slices, 1 mm isotropic voxel, time repetition = 2300 ms, echo time = 2.98 ms, and field of view = 256 mm as a basis for segmentation. We also acquired diffusion-weighted images using a spin-echo echo-planar imaging sequence (64 gradient directions, *b* value = 1000 seconds/mm<sup>2</sup>, voxel size = 2 × 2 × 2.5 mm, 52 axial slices, time repetition = 6700 ms, echo time = 89 ms, field of view = 192 × 192 mm<sup>2</sup>) plus 1 volume without diffusion weighting (*b* value = 0 seconds/mm<sup>2</sup>) at the beginning of the sequence as an anatomic reference for motion and eddy current correction.

### 2.4. Procedure

The study was approved by the local institutional review board, and all participants gave informed consent. They performed the cognitive tests in their mother tongue in a quiet setting at the hospital, and the clinicians were blind to their genetic status. All participants completed the MRI protocol. Data from 4 control participants were excluded because of technical problems during MR acquisition. Thus, the correlation analyses between brain structure and cognitive scores were performed with 34 controls.

### 2.5. Statistical methods

#### 2.5.1. Regression analyses

We normalized all cognitive scores by subtracting for each variable the mean across individuals. We first conducted linear

regression or logistic regression analyses (depending on the distribution of the data), where each outcome measure was used as the dependent variable, and the group status (control vs. aPM), the participants' age (age), and their interaction (age × group) were used as explanatory variables. To assess the potential effect of CGG repeat length in the aPM group, we then performed a second regression analysis using age, CGG, and their interaction (age × CGG) as explanatory variables. The *p* values were uncorrected.

#### 2.5.2. MRI data processing

The DTI processing has been described in detail in a previous study (Battistella et al., 2013). To sum up, data were processed and analyzed using FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Motion and eddy current corrections have been performed by registering each diffusion-weighted image to the one without diffusion weighting (*b* value = 0 seconds/mm<sup>2</sup>) using a 12-parameter affine transformation. We then computed for each voxel a diffusion tensor (Mori and Zhang, 2006) and characteristic DTI contrast parameters: fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD). We then used a novel MR unbiased data processing technique that increases tissue specificity and compensates for the effect of the spatial smoothing in voxel-based analyses (Lee et al., 2009). In this procedure, dividing the smoothed unmodulated DTI maps by the smoothed modulated segmented WM tissue map compensates the effects of the spatial smoothing. Both sets of images have been previously normalized to the Montreal Neurological Institute (MNI) space and smoothed with an isotropic Gaussian kernel (6 mm full-width half maximum). Tissue specificity is ensured by multiplication with the WM probability map.

#### 2.5.3. Correlation analyses

We recently showed that the MD and the RD maps are the most sensitive parameters to detect early WM alterations in aPM carriers when performing both group and interaction analyses (Battistella et al., 2013). FA and the related AD maps showed the same pattern of alterations, but the effect size was smaller. Given the strong relationship between RD and MD for assessing the amount of water diffusion, and between AD and FA for axonal integrity, we decided to perform correlation analyses using only the MD and the FA maps. Only cognitive measures that were significantly altered in the aPM group were subsequently used to perform linear regression with both MD and FA. The latter was performed on a voxel-by-voxel basis using age as a nuisance variable. We tested the 2 groups separately and considered significant those regions surviving *p* < 0.005 (small volume corrected) and *k* > 40 for cluster extent. Centers of gravity of the significant clusters are reported in MNI coordinates. The relationship between the Luria binary score and brain structure alterations was assessed on a voxel-by-voxel basis by comparing the MD and FA values of the premutation carriers that succeeded on the Luria test with those of the carriers that did not.

## 3. Results

### 3.1. Cognitive performance

#### 3.1.1. Verbal memory abilities

Regarding the measure of verbal short-term memory (forward digit span), no significant associations with age, group, or age × group were observed. For the CVLT task (learning score), we found a significant main effect of group (*p* = 0.004) and age (*p* = 0.006) but no age × group interaction (Table 2). The aPM carriers recalled fewer total words than the control participants across the 5 learning trials, and performances decreased with age in both carriers and controls. The lack of age × group interaction shows that the effect of age on encoding processes was similar in both groups.



**Table 2**

Results of the general linear model analysis on the different cognitive tests aimed in assessing verbal memory and executive functions in the 2 groups

Cognitive measures	aPM carriers		Controls		p Value		
	Mean	SD	Mean	SD	Group	Age	Group × age
<b>Verbal memory</b>							
Forward digit span	6.41	1.39	6.55	1.03	0.94	0.47	0.34
CVLT-learning score	56	8.72	62.53	7.25	0.004	0.006	0.52
CVLT-delayed recall	11.93	2.4	13.29	2.31	0.05	0.01	0.45
CVLT-memory decay	-1.59	1.3	-1.26	1.75	0.58	0.09	0.96
CVLT-recognition <sup>a</sup> (success rate %)	42	—	48	—	0.86	0.2	0.76
<b>Executive functions</b>							
Backward digit span	5.26	1.6	5.37	1.32	0.82	0.15	0.83
Semantic fluency	48.34	13.66	52.46	12.27	0.89	0.36	0.64
Phonemic fluency	14.19	7.19	15.03	5.86	0.71	0.36	0.94
Luria sequences <sup>a</sup> (success rate %)	56	—	88	—	0.02	0.93	0.12

Key: aPM, asymptomatic premutation; CVLT, California Verbal Learning Test; SD, standard deviation.

<sup>a</sup> Logistic regression analysis.

Similar results were found for the delayed recall score, with a marginally significant effect of group ( $p = 0.05$ ) and age ( $p = 0.01$ ) but no age × group interaction. Stratifying carriers by age below and above the median and using 2-samples *t* tests, we show that the younger carrier subgroup (mean age 36.8 years) performs worse than age-matched controls in the learning score ( $p = 0.033$ ). A similar trend is observed for delayed recall ( $p = 0.126$ ). The older subgroup (mean age 57.3 years) shows the same difference ( $p = 0.039$  for learning score,  $p = 0.172$  for delayed recall score).

Memory decay score (difference between delayed recall and the final learning trial) showed no group, age, or interaction effect. Finally, the success rate for word recognition was high in both groups, and the data distribution was essentially categorical. We therefore dichotomized the score: maximum score or failure. Logistic regression model did not show any main effect of age, group, and their interaction.

### 3.1.2. Executive functions

For the backward digit span and the verbal fluencies, the effect of our explanatory variables age, group, and their interactions in the model was not significant. The performance of aPM and control groups was similar in these tasks. Regarding the Luria sequencing, logistic regression model showed a significant effect of group ( $p = 0.02$ ) but no effect of age nor age × group interaction. The probability of success in this task was significantly lower in aPM carriers compared with the controls (Table 2).

### 3.2. Relationship between CGG repeat length and cognitive performance

Regression analyses using each cognitive score as dependent variable and CGG repeat length in the aPM group as explanatory variable failed to show any significant effect of CGG repeat length or age × CGG interaction.

### 3.3. Correlation analyses between cognitive scores and diffusion-derived measure (mean diffusivity and fractional anisotropy)

We investigated anatomic correlates for the cognitive scores showing a group effect. In the aPM group, decrease in encoding ability (CVLT learning score) was associated with alterations of MD in the WM under the right dorsolateral prefrontal cortex (DLPFC) ( $R = -0.51$ ;  $p = 0.006$ ; MNI coordinates  $x, y, z = 33, 11, 43$ ) (Fig. 1).

Additional clusters were located in the WM under the motor cortex ( $R = -0.55$ ;  $p = 0.0028$ ;  $x, y, z = 14, -16, 61$ ) and under the supplementary motor area ( $R = -0.54$ ;  $p = 0.003$ ;  $x, y, z = 15, 15, 57$ ).

Decrease in retrieval process (CVLT delayed recall) correlated with altered MD measures of left hippocampal fimbria/fornix and stria terminalis ( $R = -0.63$ ;  $p = 0.0004$ ;  $x, y, z = -30, -28, -8$ ) (Fig. 2). We did not find any significant relation between the 2 CVLT variables and MD values in the control group. FA did not correlate with any of the neuropsychological scores.

For motor planning (Luria sequencing task), we did not find any significant clusters on the whole brain analysis.

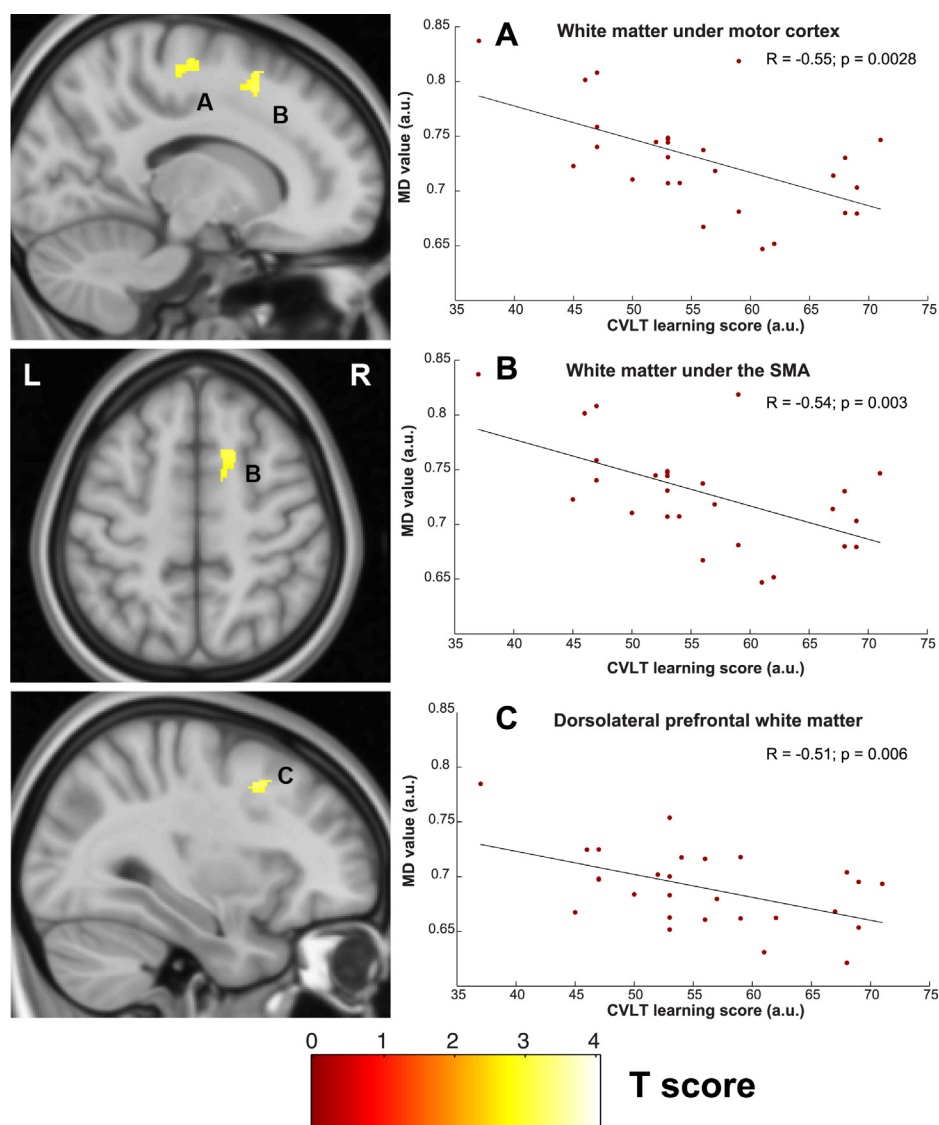
## 4. Discussion

The goal of the present study was to further investigate cognitive domains of verbal memory and executive functions while exploring brain structure in aPM male carriers. We demonstrated that aPM carriers present a decrease in verbal long-term memory in both the encoding and retrieval domains, with preserved verbal short-term memory. This memory pattern correlates with alterations of the left hippocampal WM and the DLPFC WM microstructures in aPM carriers. In the domain of executive functions, the 2 groups performed similarly on central executive and verbal initiation. They did however differ in motor planning with the aPM carriers showing a decreased performance.

Analyses of the CVLT results suggest that lower retrieval performance in the aPM group is related to weaker encoding abilities. Memory decay was similar in both groups, suggesting no greater loss of information in aPM carriers. Our results are consistent with those of Grigsby et al. (2008) who observed weaker immediate and delayed recalls with preserved memory decay in an older group of aPM male carriers (mean age 59.1 years) compared with controls. In the latter study, FXTAS participants (mean age 68.1 years) and aPM performed similarly.

Previous studies report a reduction in verbal encoding abilities in affected patients (Bourgeois et al., 2007; Grigsby et al., 2008). However, only patients with advanced FXTAS (severe tremor or balance problems interfering with activities of daily living) show further memory impairments (Bourgeois et al., 2007). In the present study, we did not find any interaction of these memory measures with age. Analyses on the stratified aPM group (above and below the median age) show that the young aPM subgroup presents altered performances compared with their age-matched controls. Our results and those of the literature suggest that these changes observed in aPM carriers are present in early adulthood and accelerated decline may occur in the late stages of the disease. These results contrast with those of Hunter et al. (2008) who did not find any differences between young aPM male carriers (under the age of 50 years) and controls on a task of verbal long-term memory. However, these data were analyzed in a composite score including verbal comprehension, a domain where premutation carriers' performances are preserved until advanced stages of FXTAS (Sevin et al., 2009). This may have masked memory differences in aPM carriers.

Retrieval process (CVLT delayed recall) is associated with specific microstructural alterations of left hippocampal fimbria/fornix and stria terminalis in the aPM group. Bilateral tissue property changes in the hippocampal fimbria/fornix were recently demonstrated in the same group of premutation carriers, and alterations of this region have been found in patients with FXTAS (Wang et al., 2012b). The presence of these alterations before the onset of FXTAS and the absence of interaction with age suggest that disruption of these structures may occur in the early developmental period (Battistella et al., 2013). The left hippocampal formation is a structure involved in both encoding and retrieval of episodic



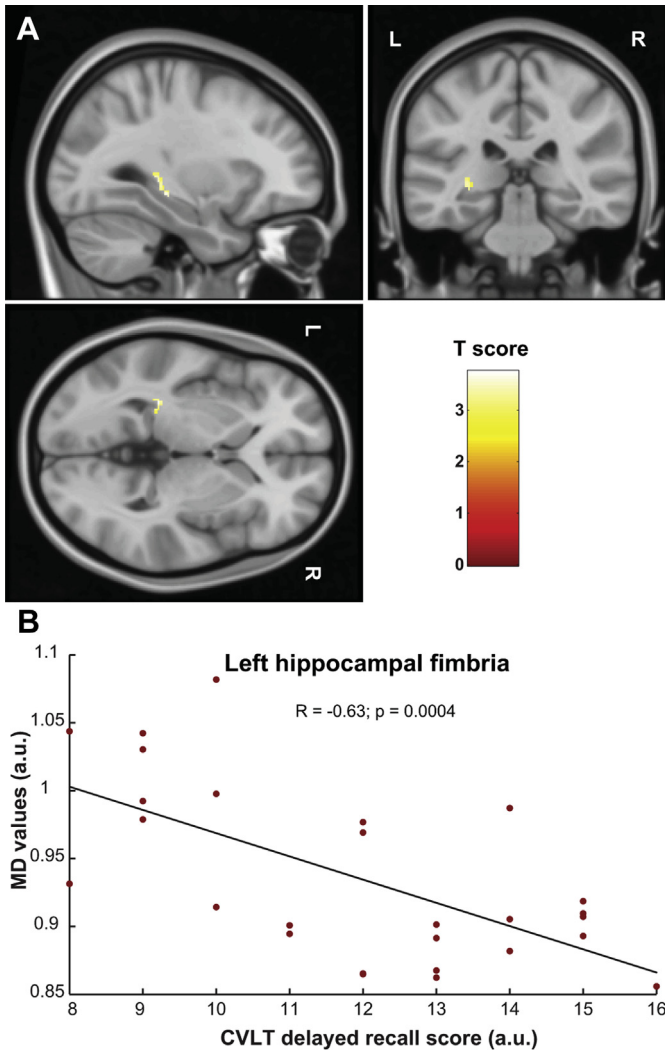
**Fig. 1.** Correlation analysis between the California Verbal Learning Test (CVLT) learning score and the mean diffusivity (MD) map. Statistical parametric maps show clusters where the MD values linearly correlate with the cognitive score. Letters label the center of gravity of the regions located in the white matter under the right dorsolateral prefrontal cortex, in white matter under the motor cortex, and under the supplementary motor area. Maps are thresholded at  $p < 0.005$  and  $k > 40$  and superposed on a standard brain in the Montreal Neurological Institute space. Hot color bar represents  $T$  scores. The rightmost side of the panel shows the linear correlation between the mean MD value in each cluster and the CVLT learning score. Corresponding Pearson correlation coefficients ( $R$ ) and  $p$  values are displayed for each plot.

information (Desgranges et al., 1998; Squire and Zola-Morgan, 1991). Left hippocampal volume has been previously found to positively correlate with the performance on verbal long-term memory tasks in premutation male carriers (Jakala et al., 1997), although authors were not able to control for the presence of FXTAS. Altered hippocampal activations, structure, and connectivity were recently reported in studies exploring visual memory (Koldewyn et al., 2008; Wang et al., 2012a) and psychological symptoms (Adams et al., 2010) in aPM male carriers. These findings suggest that hippocampal structure and function are altered before the onset of FXTAS.

Encoding process (CVLT learning score) is associated with changes in the WM of the right DLPFC in the aPM group although this region does not present structural alterations in our pre-mutation carriers. Functional MRI studies have shown that bilateral DLPFC activation is increased during verbal memory tasks requiring encoding of related items (Blumenfeld and Ranganath, 2007; Blumenfeld et al., 2011; Murray and Ranganath, 2007). This is

consistent with the CVLT that explores relational encoding through words from 4 semantic categories. The DLPFC is a structure involved in processes that support memory encoding (Blumenfeld and Ranganath, 2007), and it can therefore be related to executive domain. The lower performances observed in aPM carriers for the CVLT learning score may be because of less-efficient encoding strategies, reflecting executive weaknesses. This score correlates with 2 additional clusters located in the WM under the supplementary motor area and under the motor cortex, which are part of the executive network (Berger and Posner, 2000; Seeley et al., 2007). Of interest, these clusters show accelerated age-related changes in the aPM carriers (age  $\times$  group interaction) (Battistella et al., 2013). These results may reflect weakness in executive processes of aPM carriers and are consistent with previous findings reporting reduced activation in prefrontal regions in pre-mutation carriers with and without FXTAS (Hashimoto et al., 2011).

Regarding the executive functions measures, planning of a motor response (Luria sequencing) was significantly reduced in our



**Fig. 2.** Correlation analysis between the California Verbal Learning Test (CVLT) delayed recall score and the mean diffusivity (MD) map. (A) Statistical parametric maps show clusters where the MD values linearly correlate with the cognitive score. Maps are thresholded at  $p < 0.005$  and  $k > 40$  and superposed on a standard brain in the Montreal Neurological Institute space. Hot color bar represents  $T$  scores. (B) Linear correlation between mean MD value in the cluster located in the left hippocampal fimbria/fornix and the CVLT delayed recall score. The panel includes the scatter plot, the estimated regression line, the Pearson correlation coefficient ( $R$ ), and the corresponding  $p$  value.

aPM carriers, and there was no interaction with age or associations with anatomic correlates. However, the binary nature of the Luria score limits our power to further explore the progression of these alterations with age. To our knowledge, no studies have assessed motor planning in FXTAS or asymptomatic carriers. Deficit in imitation of motor sequencing and ideomotor apraxia have been reported in Parkinson's disease (Abbruzzese et al., 2009; Zadikoff and Lang, 2005) that shares with FXTAS similar cognitive decline. In Parkinson's patients, these deficits appear in early stages of the disease and are not related to motor severity but rather to visuospatial disability (Goldenberg et al., 1986; Zadikoff and Lang, 2005). Tasks exploring visual domains were not performed in this study, but these alterations have recently been characterized in carriers of large premutation alleles (Hocking et al., 2012). There is a growing body of research showing that a subset of carriers within the upper premutation range ( $>100$  CGG repeats) present selective executive deficits (Cornish et al., 2011; Hocking et al., 2012).

In the present study, we failed to find any changes in the aPM group for central executive and initiation of verbal response. This is likely because of low sensitivity of the tasks. Previous studies have shown that increasing working memory load is essential to detect subtle deterioration in aPM carriers (Cornish et al., 2011; Kogan and Cornish, 2010). Deficits in executive tasks involving broader network that extend beyond prefrontal cortex such as verbal fluency are observed later in patients developing FXTAS (Sevin et al., 2009) but can be found in younger premutation carriers with larger CGG (Cornish et al., 2009). Large alleles were under-represented in our aPM group (mean CGG repeats = 85.4), which may explain the absence of effect of CGG repeat length on cognitive measures. Studies have shown that individuals in the upper size range of the premutation (CGG  $>100$ ) are at higher risk of presenting age-related cognitive decline, and correlations are reported between poorer executive performance and CGG expansion in this population (Cornish et al., 2009, 2011). Recent imaging studies have shown that in addition to repeat size, messenger RNA and FMRP levels may be better predictors of the premutation-related phenotypes (Hashimoto et al., 2011; Wang et al., 2013). These measures were not available in our participants.

Despite the broad age range in our sample, we did not observe the accelerated age-related decline previously reported for certain executive tasks in aPM carriers (Cornish et al., 2008, 2009). The exclusion of 3 participants with WM lesions on FLAIR may have masked this decline in the aPM group, but including these cases in a post hoc analysis did not change the results. Analyses performed on the younger aPM subgroup suggest that verbal memory alterations are present decades before the onset of FXTAS. Longitudinal studies are required to further examine the age-related trajectory of these cognitive alterations. The 2 groups were matched on educational level, a measure that correlates with intellectual quotient (IQ). However, the lack of an IQ score is a limitation of this study as we could not control for its impact on cognitive performances. Various studies have shown that premutation carriers and controls have similar IQ range (Allen et al., 2005; Cornish et al., 2009; Grigsby et al., 2008); it is thus unlikely that memory changes and corresponding anatomic correlates are related to lower IQ in the carrier group.

In conclusion, the decrease of verbal memory and motor planning performances characterized in this study extends the pattern of early cognitive markers in premutation carriers that includes decreased central executive and visuospatial processes. We demonstrate a relationship between memory encoding scores in aPM and WM tissue property changes in the DLPFC. Memory retrieval abilities instead correlate with MD modifications in the left hippocampal fimbria, a region already shown to be altered in aPM carriers. The early onset of these cognitive and anatomic changes and their normal progression with age point toward a neurodevelopmental hypothesis.

#### Disclosure statement

The authors have no conflicts of interest to disclose.

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