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# The penetrance of marked cognitive impairment in older male carriers of the *FMR1* Gene Premutation

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## Abstract

**Background:** Male carriers of the *FMR1* premutation are at risk of developing the fragile X-associated tremor/ataxia syndrome (FXTAS), a newly recognized and largely under-diagnosed late onset neurodegenerative disorder. Patients affected with FXTAS primarily present with cerebellar ataxia and intention tremor. Cognitive decline has also been associated with the premutation but the lack of data on its penetrance is a growing concern for clinicians who provide genetic counseling.

**Methods:** The Mattis Dementia Rating Scale (MDRS) was administered in a double-blind fashion to 74 males aged 50 years or more recruited from fragile X families (35 premutation carriers and 39 intrafamilial controls) regardless of their clinical manifestation. Based on previous publications, marked cognitive impairment was defined by a score ≤ 123 on the MDRS.

**Results:** Both logistic and survival models confirmed that in addition to age and education level, premutation size plays a significant ($p<0.01$ and $p<0.03$ for logistic and survival model, respectively) role in cognitive impairment. The estimated penetrance of marked cognitive impairment in our sample (adjusted for the mean age: 63.4 years and mean education level: 9.7 years) for midsize/large (70-200 CGG) and small (55-69 CGG) premutation alleles was 33.3% (RR: 6.5; $p = 0.01$) and 5.9% (RR: 1.15; $p = 0.9$) respectively. Penetrance in the control group was 5.1%.

**Conclusions:** Male carriers of midsize to large premutation alleles had a 6-fold increased risk of developing cognitive decline and the risk increases with allele size. In addition, it was observed that cognitive impairment may precede motor symptoms. These data provide guidance for genetic counseling although larger samples are required to refine these estimates.

## Introduction

The Fragile X – associated Tremor Ataxia Syndrome (FXTAS) is a recently described late onset neurodegenerative disorder found mostly among male carriers of a premutation in the Fragile X Mental Retardation 1 (*FMR1*) gene (a repeat expansion with 55 to 200 CGG triplets in the 5'UTR of the *FMR1* gene) [1]. FXTAS also has been infrequently reported in female carriers [2] . The premutation is unstable during maternal meiosis and female carriers are at risk of having children with a "full mutation" (more than 200 CGG repeats) which is responsible for the fragile X syndrome, the most common cause of inherited mental retardation. FXTAS is defined by clinical, neuroradiological, molecular and neuropathological criteria [3-5]. Affected individuals primarily present with cerebellar ataxia and intention tremor. Less distinctive symptoms are cognitive decline or impairment, peripheral neuropathy, parkinsonism and urinary and bowel incontinence. Distinctive MRI findings include increased signals in the middle cerebellar peduncle and the deep white matter of the cerebellum [6, 7].

FXTAS is not fully penetrant and many older male carriers of the premutation may remain asymptomatic. A preliminary study by Jacquemont et al [8] demonstrated an age-related penetrance of tremor and ataxia of 17%, 38%, 47%, and 75% for male carriers of the premutation, aged 50-59, 60-69, 70-79, and over 80 years, respectively. Penetrance for different allele size is unknown. Several papers have reported the association between the older male carrier and cognitive impairment or dementia [3, 9-16]. These deficits involve executive cognitive functioning, working and declarative memory as well as inhibition and selective attention. It is unclear whether younger premutation carriers also exhibit dysexecutive symptoms and conflicting data has been published in this regard [15, 17]. Cognitive impairment and dementia may represent the most debilitating symptom affecting premutation carriers and this is a growing concern for clinicians who provide genetic counseling in fragile X families. Penetrance is essential and relevant information for proper genetic counseling to carriers of the premutation. Yet, due to the absence of data regarding the penetrance of cognitive symptoms, counseling given to premutation carriers is still vague, at best. The aim of this study is to estimate the penetrance (risk of developing symptoms) of marked cognitive impairment in older male carriers of the *FMR1* premutation regardless of their medical history. Given its prevalence (1/800 male; 1/300 female)[18, 19], the *FMR1* premutation allele likely represents the most frequent monogenic predisposition to cognitive impairment or dementia in the general population. For power issues we chose to include males only since FXTAS, cognitive impairment or cognitive decline have been infrequently reported in females[2].

## Methods

All aspects of this study were approved by the institutional review boards of the University Hospital of Nantes and the University Hospital of Lausanne CHUV.

Recruitment of premutation carriers and controls

Participants were ascertained through a family member affected with fragile X syndrome (FXS). None were referred for cognitive or neurological symptoms. Eligibility included being a male 50 years or older without a diagnosis of fragile X syndrome (FXS). Families were recruited through the FMR1 databases at the University Hospitals of Nantes, Rennes (western France) and Lausanne (Switzerland). Additional families were recruited through the local (Vendée Loire Atlantique) fragile X syndrome association. In families who accepted to participate, we identified all eligible male subjects and contacted them individually. Subjects who accepted to participate signed the informed consent form and were seen at the Center for Clinical Investigation in Nantes and in the department of neurology of Lausanne. The

recruitment of intrafamilial controls allowed avoiding stratification issues related to different cultural, education and genetic background.

Neuropsychological evaluation

This evaluation is part of a larger phenotypic study of male premutation carriers, including assessment of motor signs using standardized scales for tremor [20], ataxia [21], and parkinsonism [22] and morphologic study with MRI scans of the brain in premutation carriers.

During the evaluation, results of the genetic analyses were not available, thus the neuropsychologist administering the tests was blinded to the *FMR1* status of the participants. The evaluator would of course suspect that participants who presented overt movement disorder were carriers of a premutation allele.

*Evaluating marked cognitive impairment*

The Mattis Dementia Rating Scale (MDRS) [23] was used to assess the global cognitive status of the participants. The MDRS is a sensitive measure of impairment of different cognitive domains, and is widely used to discriminate various forms of dementia, including cortical and subcortical subtypes [24-26]. Its maximum total score is 144 and we used a cut-off score of 123 (i.e., a score equals to or below 123) on the total score of the MDRS as an indicator of marked cognitive dysfunction. This cut-off value has been shown to detect Parkinson's disease dementia (PDD) with a high sensitivity and specificity [27]. PDD is mainly characterized by frontal-subcortical impairment[28], with a neuropsychological pattern very similar to that observed in *FMR1* premutation carriers with cognitive alterations. *Other cognitive evaluations*

As prior studies suggest that patients with FXTAS have a pattern of cognitive abnormalities marked by a prominent dysexecutive syndrome [3, 11, 13], three tests providing an overview of the different components of executive cognitive functions were administered [29]: (i) the Wisconsin Card Sorting Test (WCST) to assess concept formation and mental flexibility; (ii) the phonemic and semantic verbal fluency measuring the capacity to actively generate information; and (iii) the Stroop Colour-Word Interference test, reflecting an individual's cognitive inhibition capacities. In order to assess other aspects of cognitive function, four subtests of the Weschler Adult Intelligence Scale, 3rd edition (WAIS-III) [30], were also administered: (i) Block Design, to measure visuospatial performances; (ii) Matrix Reasoning to assess nonverbal abstract reasoning capacities; (iii) Digit Span, a test of attention and verbal working memory; and (iv) the Information subtest, which is correlated to the degree of information acquired from culture. Evaluation of the visuospatial (nonverbal) working memory was made through the Corsi Blocks Task [31]. Depression and anxiety were rated by administering the Montgomery-Asberg Depression Rating Scale (MADRS) [32] and the Hamilton Rating Scale for Anxiety [33].

Molecular Analyses

DNA was isolated from peripheral blood and sizing of the CGG repeat was performed as described previously [34]. Southern blotting, used to estimate the size of alleles of more than 110 repeats (very large premutations), was performed upon *Eco*RI and *Eag*I double digestion by hybridization with StB12.3 probe. *FMR1* testing in our diagnostic laboratory is controlled by the European Molecular genetics Quality Network. Genetic results were available after completion of the cognitive evaluations: the control group was composed of individuals carrying an allele with less than 55 CGG repeats while participants with an allele size between 55 and 200 CGG repeats [35] were in the "premutation group".

Statistical Methods

Group comparison test

For group comparison we applied the Wilcoxon rank sum test to determine statistical significance between groups (controls versus carriers) and to avoid bias due to outliers.

Linear regression analysis

Data normalization was performed on all test scores as follows: for each test we subtracted the mean across all individuals and divided by the standard deviation. Bootstrapping (10,000 re-samplings) based linear regression was carried out to establish the effects of age, education and CGG repeat length on each cognitive test score. The advantage of this procedure is that it does not pose any assumption on the distribution and it is robust against outliers.

Logistic regression analysis

The continuous MDRS scores were first transformed into binary scores using the 123 cut-off, i.e. taking the value 1 if the MDRS ≤ 123, and 0 otherwise. These binary variables were then modeled according to the logistic regression framework. Here again we used bootstrapping (10,000 re-samplings) to obtain confidence intervals for the parameters.

Survival analysis

Binarized MDRS score was used as the response variable with the "survival function" defined as $S(T>age;\ CGG\ repeat\ length,\ education\ level \mid a_1, a_2, a_3) = exp(-age*(a_1 + a_2*CGG\ repeat\ length + a_3*education\ level))$, where $T$ denotes the time when the given individual develops marked cognitive impairment. Likelihood-ratio test was performed to assess the effect of CGG repeat length on the response variable.

Model evaluation

Akaike Information Criterion (AIC) was used to compare different, not necessarily nested, models.

Predicting the score for the MDRS (MATTIS dementia rating scale)

The estimated linear regression parameters were substituted back into the linear model in order to obtain estimates for the MDRS score (for a given age, education and CGG repeat length).

Predicting the penetrance of marked cognitive impairment

Logistic regression coefficients were substituted back into the logistic model in order to obtain estimates for the penetrance of cognitive impairments (for a given age, education and CGG repeat length).

Multiple testing

We applied the Benjamini-Hochberg False Discovery Rate (FDR) approach to control for multiple testing.

## Results

The recruitment process is detailed in Figure 1. The demographic data from the 75 participants (one participant had an unmethylated full mutation and was excluded) are shown in Table 1.

| | Controls | Premutation carriers | | |
|---|---|---|---|---|
| | (CGG < 55) | Small premutation (55-69) | Midsize-large premutations (70-200) | All premutations (55-200) |
| Number of patients | 39 | 17 | 18 | 35 |
| Age: median (range) | 59 (52-81) | 64 (54-80) | 66 (50-79) | 65 (50-80) |
| Education level, years: median (range) | 9 (5-20) | 9 (5-17) | 11 (5-16) | 11 (5-17) |
| CGG repeat length: median (range) | 27 (17-53) | 64 (57-69) | 83.5 (70-150) | 70 (57-150) |

Table 1: Participant's demographic and genetic status.

Age and education were not statistically different between groups (p=0.34, and 0.63, respectively). The premutation range was divided based on the previous data showing significant excess of allele's ≥ 70 CGG in Patients with FXTAS [36].

*Group and categorical analysis*

Subjects from the carrier group scored significantly worse than controls on the MDRS, a measure of global cognitive function: control group had a median of 137 (range: 108-144); carrier group had a median of 135 (range: 58-143), p=0.04 (Wilcoxon rank-sum test). The lowest cognitive performances were recorded in the carriers of midsize to large premutation alleles, which had a median of 132.5 (range: 58-143), p=0.04 (Fig. 1 of supplemental file).

*Effect of CGG repeats on the dementia score (MDRS)*

Linear regression performed on the complete data set demonstrated that CGG repeat size, age and education, each had a significant effect on the MDRS score which remained significant after controlling for multiple testing of different response variables (FDR<0.05): CGG repeat size (beta = -0.12, SE = 0.05, $CI_{95}$ = [-0.2, -0.05], p<0.002), education level (beta = 0.68, SE = 0.32, $CI_{95}$ = [0.2, 1.21], p<0.01), and age (beta = -0.48, SE = 0.19, $CI_{95}$ = [-0.81, -0.19], p<0.002). This linear model explained more than a quarter of the variance of the MDRS score ($R^2$ = 0.26). In addition, age, CGG repeat and education level are also significantly associated with the quantile-quantile normalized MDRS score. This fact excludes any bias in our results due to the non-normal MDRS score distribution (all p-values $<10^{-3}$). Other regression models, such as piece-wise linear-, quadratic- and step-functions, were also fitted to the data, but the simple linear regression yielded the best AIC value. We used this linear model to predict the distribution of the MDRS scores for controls, small and large premutation carriers at 60 and 70 years of age (Fig. 2). These distributions enable one to visualize the proportion of carriers and controls with marked cognitive impairment (visible in red on Fig. 2).

*Penetrance of marked cognitive impairment in premutation carriers > 50 years*

We defined three groups according to CGG repeat length: normal (0-54 CGG), small premutation (55-69 CGG) and midsize/large premutation (70-200 CGG). These premutation groups were defined based on previous data showing significant excess of allele's ≥ 70 CGG in Patients with FXTAS [36]. Cognitive impairment was defined as an MDRS score ≤ 123.

The estimated penetrance of marked cognitive impairment in our sample (mean age 63.5 years; mean education level: 9.7 years) for midsize/large and small premutation alleles was 27.8% (Relative Risk (RR): 5.4; p = 0.03) and 17.7% (RR: 3.4; p = 0.15), respectively (Table 2). RR values were computed relative to the risk ratio in the control group.

In order to control for the slightly unequal distribution of age and education between these groups (Table 1), we adjusted the MDRS scores for those 2 parameters using the linear regression described earlier. The adjusted penetrance (for 63.5 years of age and 9.7 years of education) increased for midsize/large alleles: 33.3% (RR: 6.5, p = 0.01) and controls (5.1%). It was slightly lower for small alleles 5.9% (RR: 1.1, p = 0.9). In both analyses, only the midsize/large alleles (≥ 70 CGG) yielded significant relative risk (6-fold) for cognitive impairment (Table 2). Our sample size was to small to provide penetrance estimates for further categories (different age groups, further CGG repeat size categories) so we modeled the effects of CGG, age and education on cognitive impairment.

| | | Penetrance of marked cognitive impairment at 63.5 y. | | ** RR (CI $_{95}$) ; p |
|---|---|---|---|---|
| | | unadjusted | * adjusted | |
| | ***controls** (0-54 CGG)* | 5.1% $_{(2/39)}$ | 5.1% $_{(2/39)}$ | 1 |
| **Premutation** | ***small** (55-69 CGG)* | 17.7% $_{(3/17)}$ | 5.9% $_{(1/17)}$ | 1.1 (0.1 – 11.8) ; 0.9 |
| | ***Midsize-large** (70-200 CGG)* | 27.8% $_{(5/18)}$ | 33.3% $_{(6/18)}$ | 6.5 (1.5 – 29.1) ; 0.01 |

Table 2. Penetrance of marked cognitive impairment stratified by CGG repeat length.
Marked cognitive impairment was defined by an MDRS score ≤ 123.
* MDRS scores were adjusted for the mean age and education level of our sample (63.5 and 9.7 years, respectively). Individuals with large CGG repeat expansions are at 6 times higher risk of developing marked cognitive impairment than those with repeat size in the normal range.
** Relative risk (RR) and CI $_{95}$ are given for adjusted data. We obtain similar results with non-adjusted MDRS scores.

*Effect of CGG repeats on marked cognitive impairment*
In order to estimate the effect of CGG repeats on cognitive impairment, the MDRS scores were binarized (score ≤ or > 123). This binary, cognitive impairment indicator score was then modeled by logistic regression including CGG repeat (beta=-0.04, SE=0.03, $CI_{95}$=[-0.12, -0.01], p<0.003), age (beta=-0.22, SE=0.19, $CI_{95}$=[-0.68, -0.07], p<0.002), and education (beta=0.21, SE=0.30, $CI_{95}$=[-0.08, 0.78], p=0.09) as explanatory variables. Naturally, dichotomizing the MDRS scores decreases the statistical power. The cognitive impairment indicator score was also modeled using survival analysis and likelihood ratio test confirmed that the CGG repeat length is significantly associated with the cognitive impairment (p<0.03). These two analyses demonstrate that the penetrance of cognitive impairment increases with the CGG repeat size.

*Cognitive decline and FXTAS may occur independently*
Based on the neurological evaluation and the rating of tremor, ataxia and parkinsonism scales, 15 participants had postural or intention tremor (4 controls and 11 premutation carriers), and 19 participants had some degree of gait impairment affecting the score of the cerebellar ataxia rating scale (4 controls and 15 premutation carriers). Two out of the 8 premutation carriers with marked cognitive alterations had no sign of movement disorder at the time of the examination. A third case was followed during several months for a rapidly progressive isolated dementia before the onset of any motor sign. The remaining 5 carriers with a cognitive disorder had clear motor symptoms, fulfilling the diagnostic criteria for probable or definite FXTAS [3, 4]. Brain MRIs, performed in 29 /35 premutation carriers, found periventricular and subcortical white matter abnormalities in 8 cases, and increased T2 signal in the middle cerebellar peduncle in 7 cases. Both radiological abnormalities were found in 3 carriers with cognitive alterations.

*Cognitive decline or Cognitive impairment*
In order to differentiate between cognitive decline and cognitive impairment in this cross sectional dataset, we compared the regression slope of age-related cognitive decline in the

control and premutation groups (Fig. 3). Both negative slopes indicated significant and inverse association of MDRS with age (p=0.03, p=0.05, respectively) but the rate of cognitive decline was not significantly different between the two groups (p=0.22). The rate of decline for the subgroup of midsize to large alleles (≥70 CGG) was faster but the difference with controls remained non significant (p=0.17). Increased variance in the MDRS scores was also seen in the older carriers (>65y.) in comparison to the younger ones (<65y.) ($p<10^{-6}$), illustrating the fact that a subgroup of carriers presents significant cognitive impairment while others function normally. All in all, given the large amount of noise and our modest sample size we are statistically underpowered to demonstrate that premutation carriers exhibit a faster cognitive decline than controls although such trend is visible.

We controlled for the potential effect of depression on cognitive function[37, 38] as well as for familial clustering (c.f. Supplemental Files).

*Characterization of the cognitive impairment*

Linear regressions demonstrated that CGG repeat size was significantly altering three subscores of the MDRS, as well as the perseverative errors rate of the WCST, semantic fluency, abstract reasoning and visuospatial performances (Matrix reasoning and Block design subtests of the WAIS-III). In all these analyses, a larger premutation allele was associated with poorer cognitive performances. The working memory tasks (Digit span and Corsi Blocks), and the Information subtest of the WAIS-III were not influenced by the CGG repeat size (Fig. 4 and Supplemental file Table 1).

## Discussion

To our knowledge, this is the first study evaluating the penetrance of cognitive impairment in older male carriers of the *FMR1* premutation. In our sample, the penetrance for carriers of midsize-large premutation alleles (≥70 CGG repeats) was six times higher (p=0.01) than in controls (33.3% *vs* 5.1%). There was no increased risk for small premutation alleles (55-69 CGG repeats) compared to controls after adjusting for age and education (Table 2). These penetrance estimates apply to a carrier with an age of 63.5y and relatively low level of education (9.7 years). The regression models do predict an elevated risk for small premutation alleles at a later age (e.g. 70y – Fig. 2) but one should avoid strong conclusions obtained via extrapolation to an age range considerably higher than our sample mean.

Determining a cutoff score associated with significant cognitive impairment or dementia is still a matter of debate. Some authors have established this limit between 132 and 129 on the MDRS[39, 40]. Using 129 as an alternative cutoff, we obtained of course higher penetrances: 38.9% (RR 3.8), 11.7% and 10.2% for midsize/large, small and normal alleles respectively. MDRS scores between 129 and 124 denote mostly mild cognitive alterations, with minimal functional impact. The more stringent cutoff of 123 reported by Llebaria et al [27] to screen dementia in PD is therefore more appropriate for genetic counseling purposes. Aarsland et al. reported mean MDRS score of 120.6 (SD: 7), 118 (SD: 10.8) and 125.7 (SD: 5.3) in groups of patients with mild to moderate dementia and a diagnosis of PD, dementia with Lewy bodies and progressive supranuclear palsy respectively[24]. Over half of these patients with dementia had MDRS scores > 120 which is an additional argument in favor of a cutoff slightly higher than 120.

We compared our control group to previous normative studies of the MDRS in the general population.[41, 42] Frequencies of 3-5% [41] have been reported for marked cognitive impairment (using an MDRS score ≤ 123) in a male population (n=166) aged 69-71 years old with an education level of 13.1 years. [41] Using our logistic regression model to adjust our control

group for the same age range and education level, we obtained very similar penetrance of marked cognitive impairment: 4.8%. Our sample shows a predominance of small to midsize premutations alleles (Fig. 2, Supplemental file), which is closer to the distribution in the general population and a sign of limited ascertainment bias [36, 43]. Our sample did not properly cover the very large allele range and the penetrance in this latter category is likely to be significantly higher.

It is unclear whether premutation carriers have an accelerated age-related decline or if they present, earlier on with lower MDRS scores. Our premutation group declined faster than controls but this difference was not statistically significant (Fig. 3). Our sample size is too small to clearly answer this question. Conflicting data has been published on the presence of executive function deficits in younger premutation carriers. Hunter et al.[17] reported no effect of CGG repeats on neuropsychological performance in males < 50 years of age even when only very large alleles (>100 CGG) were taken into account. On the other hand, Cornish et al[15] found deficits in response inhibition and working memory in premutation carriers in their forties. Moderate to severe symptoms are likely the result of interactions between the premutation allele and other genetic or environmental factors, as we clearly see increased variance in MDRS scores for older carriers in comparison to younger ones ($p<10^{-6}$).

Two out of the 8 carriers of a premutation with marked cognitive alterations had no sign of movement disorder at the time of the examination. This indicates that cognitive impairment and dementia may precedes the onset of motor symptoms in many cases, and supports previous reports [44, 45]. Cognitive impairment is not always obvious to family members since the onset of cognitive alteration is often insidious and may be noted only when overt disability occurs.

The profile of neuropsychological alterations observed in our sample is in accordance with previous studies [11, 14, 15]. It confirms that executive dysfunction is the core feature of the cognitive impairment in premutation carriers, associated with impairment of memory and visuo-spatial processing, a pattern typically observed in "subcortical" dementias, such as Parkinson's disease dementia [28]. In contrast to results reported by Grigsby et al. [13] (who mainly evaluated carriers affected with FXTAS), our sample did not show evidence of impairment in working memory tasks (Corsi blocks and digit span). However, the memory subscore of the MDRS, which includes items assessing long-term and short-term memory, was correlated to the CGG repeats length. These results suggest that the premutation might affect long-term memory, a feature that should be specifically investigated.

We recommend evaluating older male carriers for executive function deficits as well as depression and anxiety symptoms. If present, medications for cognitive deficits have anecdotally been reported to be helpful in patients with FXTAS. The genetic counseling priority in these families should remain the information and prevention of fragile X syndrome by screening women of child bearing age at risk of transmitting the Fragile X syndrome. By doing so, clinicians will often perform involuntary presymptomatic testing in males identified as obligate carriers. Symptoms have a later onset and their penetrance is lower than in other genetic neurodegenerative disorders such as Huntington's disease. A rigid protocol for presymptomatic testing may therefore not always appropriate. Our data on the penetrance of cognitive impairment will help clinicians provide the necessary information before they propose premutation screening in these families.

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**Figure legends**

Figure 1. Recruitment Process.
* includes also potential participants who were not informed of the study due to family miscommunication.
** full mutation (unmethylated): excluded from analysis.

Figure 2. Predicted distribution of MDRS score.
Each panel represents the predicted distribution of the MDRS total score, based on the linear regression model of the data: Controls (30 CGG repeats) aged 60 (a) and 70 years (b). Short premutation carriers (60 CGG) at 60 (c) and 70 years of age (d). Large premutation carriers (90 CGG) at 60 (e) and 70 years of age (f). For all estimates we set the education to 9.5 years, which was the mean education level in our study. The vertical line indicates the cut-off score for marked cognitive impairment (123 on the MDRS). In this model, elevated penetrance of cognitive decline for small premutation carriers becomes apparent after the age of 65 years.

Figure 3. Data visualization.
Individual MDRS scores (corrected for education, and set to the mean education level of the sample, 9.5 years) were plotted against age for both controls and premutation carriers. Regression slopes indicate the age-related decline in the MDRS score. A faster, albeit not statistically significant (p=0.22), age-related decline (slope) can be observed for premutation carriers compared to the control group.

Figure 4: CGG Repeat Regression Coefficients for Cognitive Assessments.
Blue squares mark the median value and whiskers represent the 95% confidence interval. Bold whiskers, that do not overlap the red vertical line (value of 0), represent significant coefficients ($p<0.05$). CGG has a significant impact on MDRS total score, and on 9 out of 26 other scores and subscores. Most scores decrease as they worsen, thus most coefficients are negative (as CGG repeat increases, scores worsen and drop). For "% perseverative errors", "items for first category", "Hamilton", and "MADRS", increasing scores denote pathological results. Those coefficients were indicated by black cross next to the test name and inverted so they are presented in the same direction as the other negative coefficients. Red star next to the test name marks the scores which remained significant with FDR<0.05 after Benjamini-Hochberg multiple testing correction. Confidence intervals that do not contain zero are marked with thicker lines.

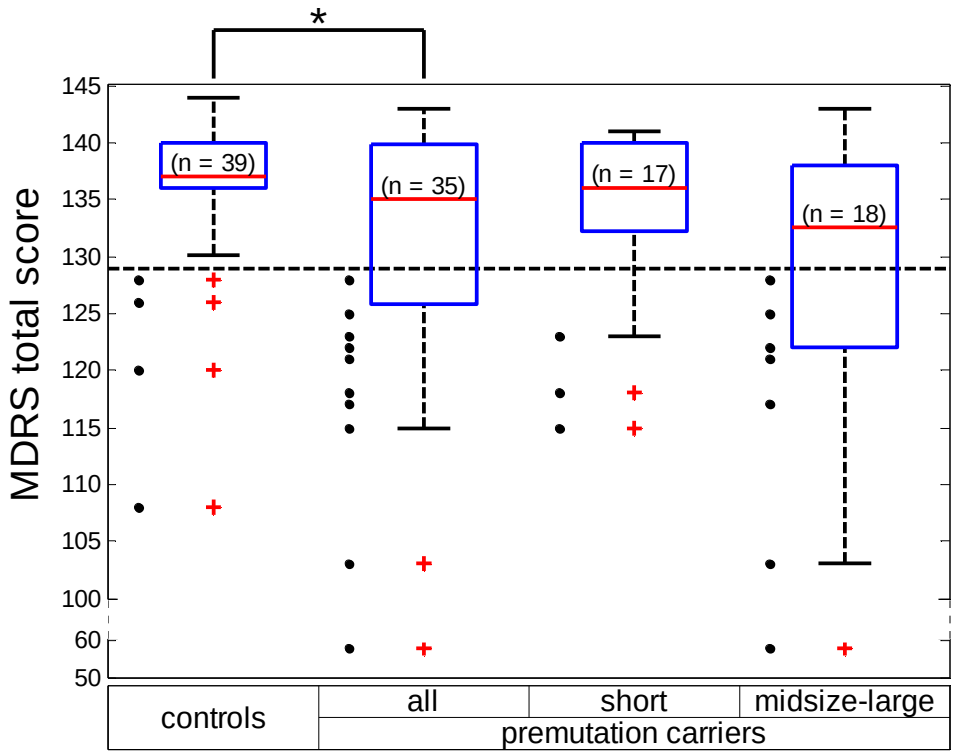
# Supplemental files

*Controlling the effect of depression on cognitive function*

The Montgomery-Asberg Depression Rating Scale (MADRS) was significantly influenced by the CGG repeat size. To prevent a bias due to the impact of depressive mood and anxiety on cognitive performances, we added the MADRS and Hamilton scores as additional covariates (besides the age, CGG repeat and education) to our regression model for the MDRS score. CGG repeat (beta=-0.21, SE=0.11, $CI_{95}$=[-0.44, 0], p=0.05), age (beta=-0.29, SE=0.12, $CI_{95}$=[-0.53, -0.07], p<0.01) and education (beta=0.15, SE=0.10, $CI_{95}$=[-0.05, 0.34], p=0.11) still had at least marginally significantly non-zero coefficients, while depression scores did not significantly contribute to the MDRS (p>0.2). Thus, the MDRS score is – among the measured parameters – primarily driven by CGG repeat, age and education, and not by depression or anxiety.

*Controlling for the effect of familial clustering on cognitive function*

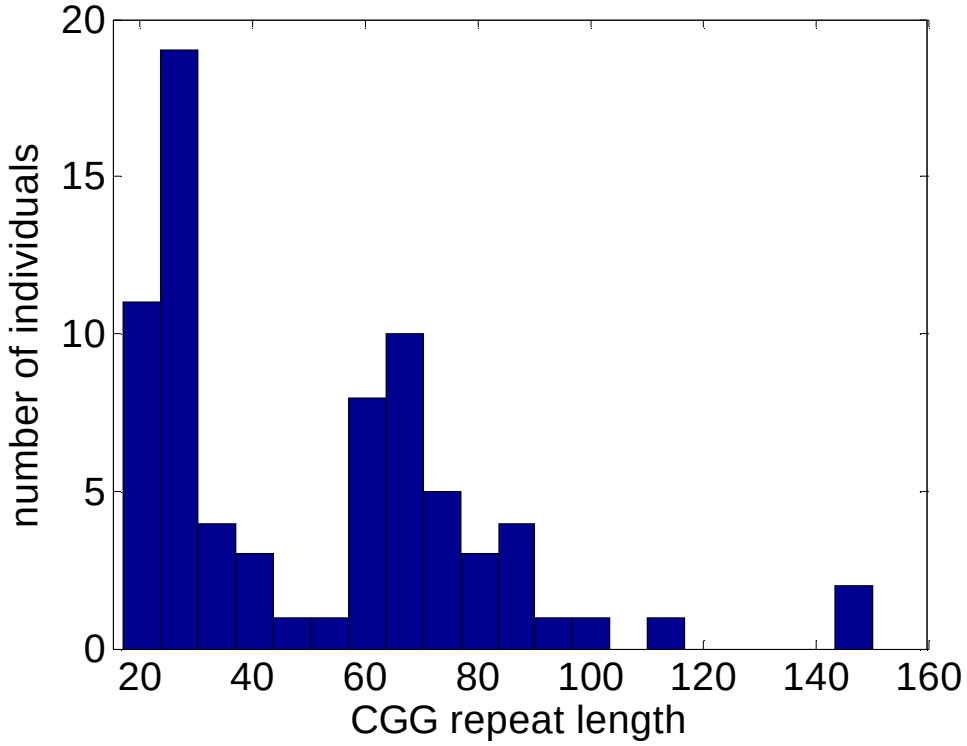
We found no association between familial background and MDRS score (ANOVA p=0.18). Furthermore, our regression results do not change when familial backgrounds are added as covariates.




Supplemental file, Figure 1: Boxplots Showing the Distribution of MDRS Uncorrected Total Score in the Premutation and Control Groups.

The premutation carrier group was subdivided based on CGG repeat size. The red midline of the box denotes the median, the extremes of the box the inter-quartile range, and the bars the upper and lower limits of the 95 percent confidence interval. The crosses denote outlying values. On the left of each bar the individuals in the given group whose score is below 129 are marked with black dots. The horizontal dotted line indicates the 129 cut-off value for the MDRS.

* : $p<0.05$

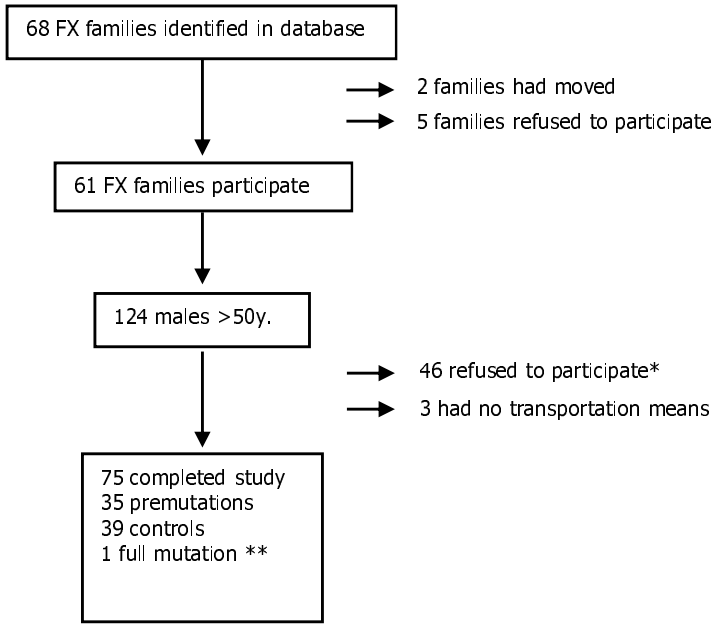



Supplemental file, Figure 2: CGG repeat length distribution.

| Dependent variable | CGG repeat length regression coefficients | | | |
|---|---|---|---|---|
| | beta | SE | $CI_{95}$ | p-value |
| **MDRS total** | **-0.2926** | **0.1193** | **[-0.56, -0.10]** | **0.0016** |
| MDRS attention | 0.0161 | 0.1193 | [-0.19, 0.28] | 0.9434 |
| **MDRS verbal initiation** | **-0.3659** | **0.1139** | **[-0.57, -0.12]** | **0.0072** |
| MDRS motor initiation | -0.1126 | 0.1398 | [-0.44, 0.09] | 0.4792 |
| MDRS construction | -0.1111 | 0.1442 | [-0.45, 0.08] | 0.6282 |
| **MDRS concepts** | **-0.2181** | **0.1281** | **[-0.51, -0.01]** | **0.0354** |
| **MDRS memory** | **-0.2213** | **0.129** | **[-0.51, -0.01]** | **0.0348** |
| STROOP colors | -0.1467 | 0.1367 | [-0.38, 0.16] | 0.2874 |
| STROOP words | -0.2289 | 0.1255 | [-0.46, 0.04] | 0.0828 |
| STROOP interferences | 0.1517 | 0.1628 | [-0.07, 0.55] | 0.3534 |
| **WCST % perseverative errors** | **0.3145** | **0.1248** | **[0.12, 0.60]** | **0.0008** |
| WCST nr conceptual answers | -0.1881 | 0.1436 | [-0.52, 0.05] | 0.1416 |
| WCST nr categories | -0.1753 | 0.1035 | [-0.40, 0.01] | 0.0688 |
| WCST items for 1st category | 0.2113 | 0.1428 | [-0.01, 0.55] | 0.0648 |
| Phonemic verbal fluency | -0.1622 | 0.1122 | [-0.39, 0.06] | 0.145 |
| **Semantic fluency** | **-0.2881** | **0.1004** | **[-0.48, -0.08]** | **0.0076** |
| digit span Total | -0.0689 | 0.161 | [-0.35, 0.28] | 0.6078 |
| digit span Direct | -0.0215 | 0.1428 | [-0.27, 0.30] | 0.815 |
| digit span Indirect | -0.1858 | 0.1499 | [-0.48, 0.11] | 0.2096 |
| Corsi block Total | -0.1197 | 0.1177 | [-0.34, 0.13] | 0.291 |
| Corsi block Direct | -0.1141 | 0.1235 | [-0.33, 0.15] | 0.3402 |
| Corsi block Indirect | -0.1542 | 0.1094 | [-0.39, 0.05] | 0.1384 |
| **Block design – visuospatial** | **-0.2329** | **0.1168** | **[-0.47, -0.01]** | **0.0342** |
| **Matrix – abstract reasoning** | **-0.2077** | **0.101** | **[-0.42, -0.02]** | **0.0266** |
| Information | -0.0395 | 0.1117 | [-0.28, 0.16] | 0.7672 |
| **MADRS - depression** | **0.3577** | **0.1999** | **[0.01, 0.75]** | **0.043** |
| Hamilton – anxiety | 0.2477 | 0.1408 | [-0.00, 0.56] | 0.0516 |

Supplemental file, Table 1. Regression coefficients for CGG repeat length for various dependent variables. Bold characters represent significant coefficients. Other explanatory variables were age and education. Figure 4 is the visualization of this table.

Figure 1.



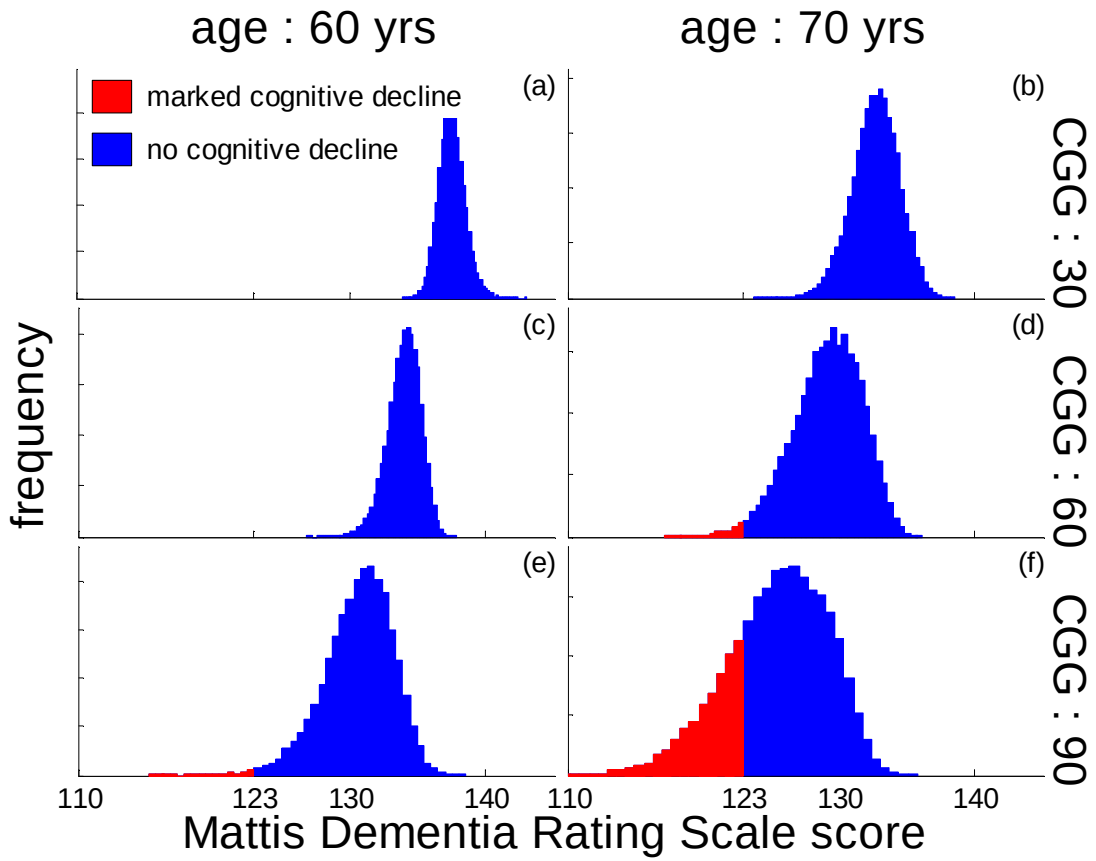



Figure 2.

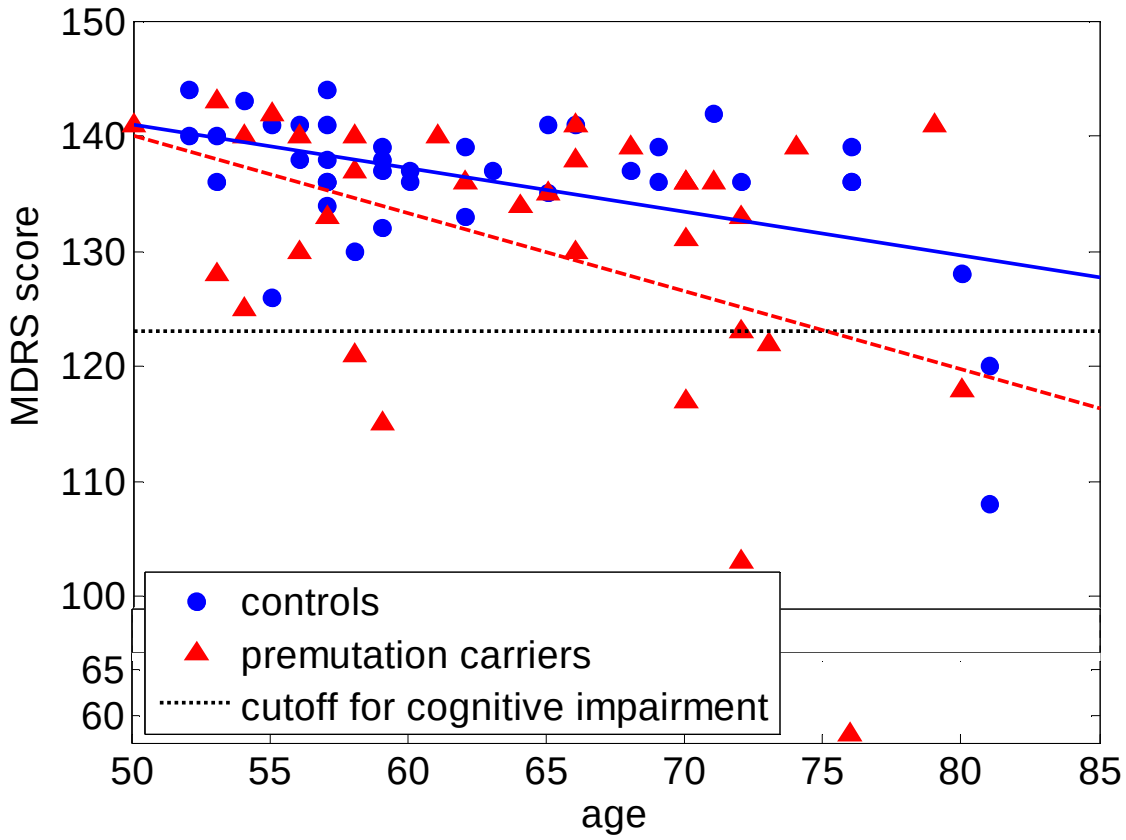



Figure 3.

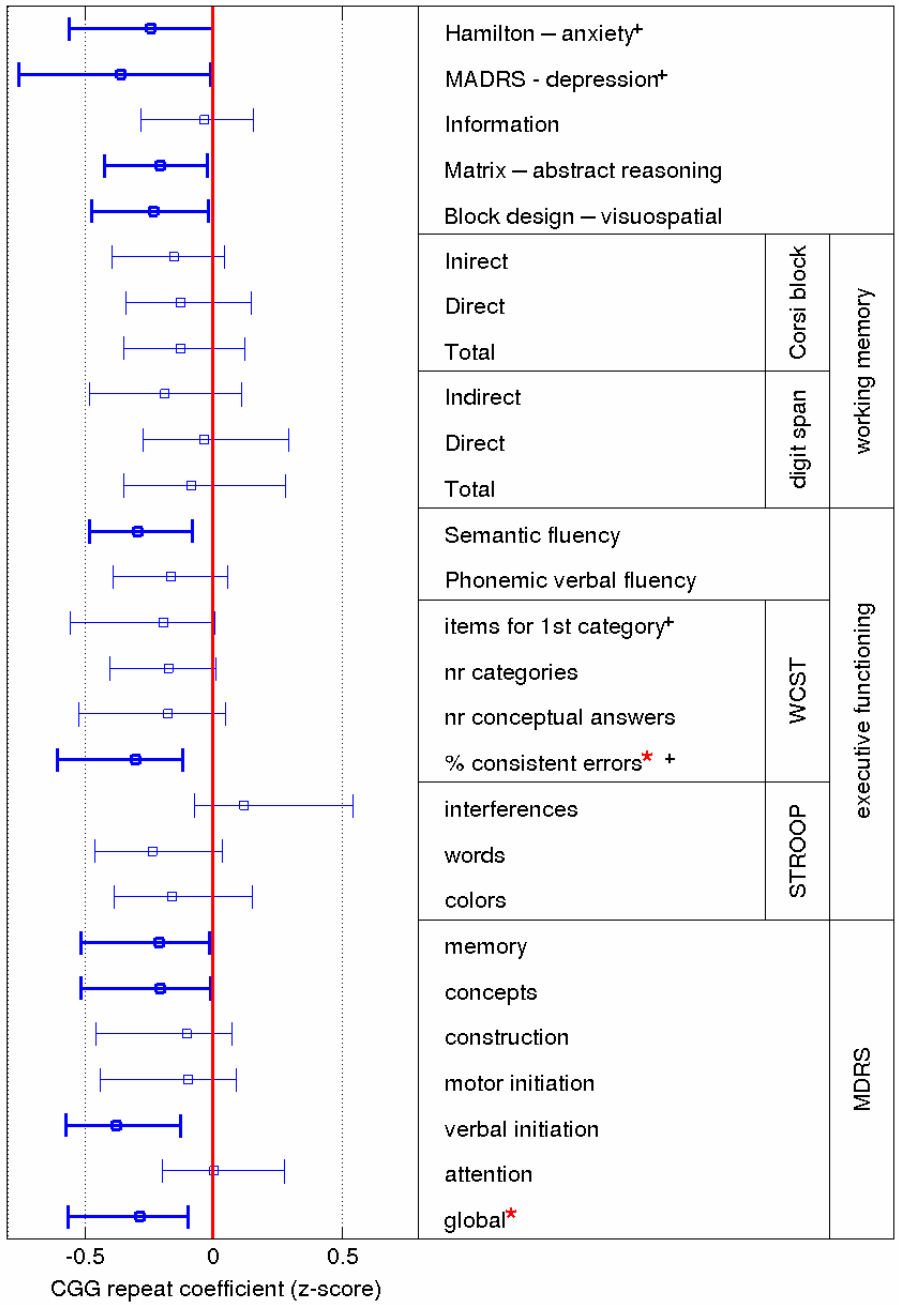



Figure 4.