

Paper VI

***FMRI* alleles in Parkinson's disease: Relation to cognitive decline and hallucinations. A longitudinal study**

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ABSTRACT

Carriers of expanded alleles of the fragile X mental retardation (*FMRI*) gene may display parkinsonism, cognitive decline and behavioral changes. We screened two male groups of patients affected with Parkinson's disease (PD) (n=137). One group (n=56) was followed longitudinally for up to 12 years. Length of CGG repeats in PD subjects was compared with healthy controls (n=310). In addition, the association of the number of CGG repeats with cognitive decline or hallucinations was studied in the longitudinally followed PD group. We found no repeats in the premutation range (55–200 CGG repeats) and no significant difference in the proportion of intermediate-size (41-54 CGG repeats) carriers between the PD and the control groups. Using linear regression, the number of CGG repeats was not related to motor or cognitive progression. However, the marked cognitive decline in two patients carrying intermediate-size alleles point to a possible association. More studies with larger PD samples are warranted.

Introduction

Parkinsonism is one of the clinical features of fragile X-associated tremor/ataxia syndrome (FXTAS) and it has been reported that probable FXTAS occurs in 39% of male carriers of expanded CGG repeats of the *FMRI* gene (full mutation >200 CGG repeats).¹ CGG repeats in the premutation range of the *FMRI* gene (55–200 CGG repeats) may furthermore be associated with cognitive and behavioral disturbances in older men and there exists evidence that repeat numbers in the intermediate-size range (41-54 CGG repeats)² or even in the high end of the normal range (35-40 CGG repeats) may play a pathogenic role.³

Cognitive and behavioral disturbances are common in Parkinson's disease (PD),⁴ and thus indicate possible influence of premutations or intermediate-size alleles of the *FMRI* gene. However, premutations were not found more often in PD than in the general population.⁵ To our knowledge, no studies have yet explored the relationship between CGG allele size of the *FMRI* gene and dementia in PD. Since cognitive decline and visual hallucinations increase with disease duration, a continuously design is crucial to explore a potential relationship with genetic factors. Therefore, in a continuously study we explored whether CGG allele size was associated with PD and with cognitive decline and visual hallucinations in PD.

Materials and Methods

Patients and controls

The PD group consists of 137 male patients whereof 56 were drawn from a longitudinal study in Stavanger (STV), Norway⁴ (follow-up time 4 to 12 (mean 9.6) years) and 81 were drawn from an outpatient clinic from the Ruhr area (RA), Germany. Ethnically matched control groups of healthy blood donors were recruited in Bergen, Norway (n=171) and RA, Germany (n=139) (mean age of German controls: 43 years, range 19 – 69 years). This study was approved by the local ethics committees.

Evaluation programme

Diagnosis of PD

The diagnostic evaluation in both groups was based on a neurological examination and clinical information about onset of disease, disease development, and the response to levodopa according to definite criteria.⁶ Autopsy of 22 deceased patients from the continuously followed group revealed brain changes compatible with a diagnosis of PD.⁷

Clinical evaluation

In the continuously followed group, parkinsonism was rated using the motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS).⁸ Cognitive functions were rated using the Mini-Mental State Examination (MMSE),⁹ and hallucinations with the Thought disorder item of the Mental subscale of the UPDRS.⁸ A score of 2 and above was defined as visual hallucinations. Marked cognitive impairment indicating dementia was defined as a MMSE score below 20. Patients from the RA group were only studied in respect of *FMRI* allele size. In this group appropriate follow-up data were incomplete and patients were included in the study to allow a better association of number of CGG repeats of the *FMRI* allele with the diagnosis of PD.

Molecular analyses

After receiving informed consent, blood samples were drawn, and genomic DNA was extracted according to standard protocols. The size of the individual CGG repeats was determined by polymerase chain reaction (PCR) as follows: For each PCR reaction, a 10 µl reaction mix was set up containing 100 ng DNA, 1x GC buffer (Genecraft, Münster, Germany), 1 U Taq Polymerase (Genecraft, Münster, Germany), 0.2 mmol of each dNTP, 0.4 mmol of each primer (forward primer 5'-Cy5-CGCTCAGCTCCGTTTCGGTTTCACTTCCGGT-3'; reverse primer 5'-TCCTCCATCTTCTCTTCAGCCCT-3') and varying concentrations of MgCl₂ (1-3 mmol; Qiagen, Germany). PCR was performed with an initial denaturation at 95°C for 15 min, 35 cycles of 95°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 5 min. Repeat length was analyzed on a DNA sequencer using a 400 DNA standard (Beckmann Coulter Ceq 8000).

Statistical analyses

To address a potential relationship between the number of CGG repeats and the clinical features in PD the Norwegian PD patients from the ongoing study were categorized in two groups according to the intermediate size range (i.e. 41-54).

Annual decline on MMSE was calculated as the difference between the last score before decline began and the last score (or the first time a score of 0), divided by the number of years between these two scores. To analyze whether clinical characteristics differed according to the number of CGG repeats, linear regression analyses were performed, using annual change on MMSE and UPDRS III as the dependent variable, and allele size and age as independent variables. A p-value of <0.05 was chosen as statistically significant.

Results

No premutation alleles were found. In the STV group 2 PD patients (3.6%) and 2 controls (1.2%) had 41-54 CGG repeats ($p=0.63$); in the RA group 5 PD patients (6.2%) and 3 controls (2.2%) had 41-54 CGG repeats ($p=0.125$). The proportions of CGG repeats in the groups are shown in table 1.

In the longitudinally studied STV group, the regression analyses did not show any associations between change in UPDRS III ($p=0.9$) and MMSE ($p=0.17$) with number of CGG repeats. These results did not change after including baseline MMSE and UPDRS scores as independent variables in addition to age. The clinical characteristics of PD patients with less than 41 and the two PD patients with 41 or more CGG repeats are shown in table 2. There were no clinical differences at baseline. However, both patients with greater than 41 CGG repeats developed a syndrome of severe dementia and marked hallucinations during the course of disease. They declined 2.33 and 3.22 points per year respectively, compared to a mean (SD) decline of only 1.4 (range 0-3.8) in patients with less than 41 repeats.

Discussion

FXTAS is believed to be a relatively common disorder affecting up to 1 in 3000 men older than 50 years in the general population.¹⁰ FXTAS shares clinical features with MSA and parkinsonism.¹¹ However, the phenotype of FXTAS does not often resemble typical PD when diagnosed according to standard diagnostic criteria.⁵ Consistent with these findings, none of the screened PD-patients or control subjects carried alleles within the premutation range emphasizing diagnostic accuracy in our patient groups.

The relationship between cognitive impairment and number of CGG alleles has not been investigated in a prospective followed PD cohort before. CGG repeats within the intermediate zone area may influence disease progression³ and may be associated with other diseases showing possible pathogenic relevance.² The mechanisms by which CGG-repeats in the intermediate-size range may exhibit a pathogenic role remain elusive. However, *FMRI* allele sizes in this range are associated with slightly increased *FMRI* mRNA levels^{12,13} and a gradient pathogenic risk has been suggested from an allele size for alleles beyond the normal range of ~30 CGG-repeats.³ A number of possible pathogenic mechanisms are suggested: excess *FMRI* mRNA causes intranuclear inclusions in the brains of mice with expanded CGG-repeats^{14,15} and cause degeneration of neuronal cells in *Drosophila*.¹⁶ These findings led to a gain-of-function model suggesting that the degree of degeneration is related to the relative molar quantity.¹⁷

The number of intermediate-size carriers in our groups is too low to find statistically significant associations. However, interestingly, both patients carrying intermediate size alleles developed marked dementia and hallucinations, suggesting a possible association between CGG repeats and cognitive decline in PD. Although clinical characteristics at baseline were similar in patients with intermediate-size alleles and those without both patients

carrying alleles >41 CGG repeats displayed a higher age at onset and age at baseline. As higher age emerges as risk factor for dementia and hallucinations in PD^{18,19} the difference in age may have influence in cognitive performance in our patient sample.

Clearly a longitudinal approach is needed to determine a possible relationship between CGG-repeat length and development of dementia and hallucinations in PD. The longitudinal design over a 12-year period, a population-based group, a diagnosis according to published diagnostic criteria⁶ with neuropathological confirmation in a subgroup of the patients⁷ are undisputable strengths of the study. However, the small sample with a low statistical power precludes firm conclusions. Thus, longitudinal studies with large patient cohorts are needed to further address this issue. Cognitive deficits in PD are dominated by executive dysfunction, which is not adequately addressed by the MMSE. Thus, the future studies should use more detailed neuropsychological assessments to detect the cognitive decline in PD.

The etiology of PD remains largely unknown. A complicated interaction of individual, environmental and genetic components in the etiology of PD has been suggested²⁰ and there is some evidence of a genetic contribution to dementia in PD.²¹ A dose-dependent nature of multiplication in the α -synuclein mutation similar to the gradient pathogenic risk in *FMRI* has been described in PD.²² A similar model may explain influence of CGG-repeats of the *FMRI* gene and is in line with recent findings suggesting that alleles within the intermediate range and at the high end of the normal range might have pathogenic relevance in neurodegenerative^{23,24} or other diseases.^{2,3} We propose a complex interplay with several genetic and environmental factors in the etiology of cognitive impairment in PD, and relatively large number of CGG repeats may be one of the factors. However, further investigations are needed to test this hypothesis and to explore the potential underlying processes at the molecular level involved.

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Table 1. Comparison of CGG repeat length of the *FMR1* gene between the different groups.

CGG repeat length	STV group	STV controls	RA group	RA controls
10 – 20 CGG repeats	0	30	2	12
21 – 25 CGG repeats	15	23	14	18
26-30 CGG repeats	24	66	45	60
31-35 CGG repeats	14	49	11	39
36-40 CGG repeats	1	1	4	7
41-45 CGG repeats	1	2	4	3
46-47 CGG repeats	1	0	1	0
Σ	56	171	81	139

Note. Numbers account for CGG repeats, STV group = Stavanger PD group, STV controls = Stavanger healthy controls, RA group = Ruhr area PD group, RA controls = Ruhr area healthy controls;

Table 2. Comparison of clinical characteristics at baseline and follow up between patients with ≥ 41 or < 41 CGG repeats (longitudinally followed STV group).

	Patient 1	Patient 2	<41 repeats
<i>Baseline</i>			
N	1	1	54
CGG-repeats	41	46	29.1 (3.8)
Age at onset, y	64	70	59.9 (8.9)
Age at baseline, y	71	73	67.0 (8.2)
UPDRS III	19	27	22.5 (14.2)
MMSE	29	30	27.7 (4.5)
VH	No	No	10.2%
<i>Follow up</i>			
Duration of follow-up, y	10	6	9.4(1.5)
Annual decline in UPDRS	5.6	2.17	3.6 (2.2)
Annual decline in MMSE	3.22	2.23	1.4 (1.3)
Last MMSE score	0	16	18.7 (10.3)
VH during course	Yes	Yes	55.3%

Note. Numbers represent mean (standard deviation SD) or proportion (%). MMSE = Mini-Mental State Examination; UPDRS = Unified Parkinson's Disease Rating Scale, motor subscore; VH = visual hallucinations;

