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The fragile X tremor ataxia syndrome in the differential diagnosis of multiple system atrophy: data from the EMSA Study Group

C. Kamm,[1,*] D. G. Healy,[7,*] N. P. Quinn,[8] U. Wüllner,[2] J. C. Moller,[3] L. Schols,[1] F. Geser,[11] K. Burk,[4] A. D. Børglum,[12] M. T. Pellecchia,[13] E. Tolosa,[14] F. del Sorbo,[15] C. Nilsson,[16] O. Bandmann,[10] M. Sharma,[1] P. Mayer,[1] M. Gasteiger,[5] A. Haworth,[7] T. Ozawa,[7] A. J. Lees,[7] J. Short,[9] P. Giunti,[7] E. Holinski-Feder,[5] T. Illig,[6] H. E. Wichmann,[6] G. K. Wenning,[11] N. W. Wood[7] and T. Gasser[1] for the European Multiple System Atrophy (EMSA) Study Group

[1]Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, [2]Gene Bank Parkinson Germany Bonn, Department of Neurology, University of Bonn, Bonn and Parkinson Competence Net e.V. (BMBF 01G19901), [3]Department of Neurology, Philipps University, Marburg, [4]Department of Neurology, University of Ulm, [5]Center of Medical Genetics, Munich, [6]GSF National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg, Germany, [7]Department of Molecular Neuroscience and [8]Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, [9]Department of Medical Genetics, St George's Hospital, Tooting, London, [10]Academic Neurology Unit, Division of Genomic Medicine, University of Sheffield, Sheffield, UK, [11]Department of Neurology, University of Innsbruck, Innsbruck, Austria, [12]Institute of Human Genetics, University of Aarhus, Aarhus, Denmark, [13]Department of Neurological Sciences, University Federico II, Napoli, Italy, [14]Neurology Service, Institut Clinic Malaltias del Sistema Nervios, Hospital Clinic Universitari, University of Barcelona, Barcelona, Spain, [15]Istituto Nazionale Neurologico 'C. Besta', Milano, Italy and [16]Neuroscience Department, University of Lund, Lund, Sweden

Correspondence to: T. Gasser, Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, Hoppe-Seyler-Strasse 3, University of Tübingen, 72086 Tübingen, Germany
E-mail: thomas.gasser@med.uni-tuebingen.de
*These authors contributed equally to this work

The recent identification of fragile X-associated tremor ataxia syndrome (FXTAS) associated with premutations in the *FMR1* gene and the possibility of clinical overlap with multiple system atrophy (MSA) has raised important questions, such as whether genetic testing for FXTAS should be performed routinely in MSA and whether positive cases might affect the specificity of current MSA diagnostic criteria. We genotyped 507 patients with clinically diagnosed or pathologically proven MSA for *FMR1* repeat length. Among the 426 clinically diagnosed cases, we identified four patients carrying *FMR1* premutations (0.94%). Within the subgroup of patients with probable MSA-C, three of 76 patients (3.95%) carried premutations. We identified no premutation carriers among 81 patients with pathologically proven MSA and only one carrier among 622 controls (0.16%). Our results suggest that, with proper application of current diagnostic criteria, FXTAS is very unlikely to be confused with MSA. However, slowly progressive disease or predominant tremor are useful red flags and should prompt the consideration of FXTAS. On the basis of our data, the EMSA Study Group does not recommend routine *FMR1* genotyping in typical MSA patients.

Keywords: multiple system atrophy; FXTAS; fragile X; *FMR1*; premutation

Abbreviations: EMSA-SG = European Multiple System Atrophy Study Group; *FMR1* = fragile site mental retardation 1 gene; FXTAS = fragile X tremor ataxia syndrome; KORA = Cooperative Health Research in the Region of Augsburg; MSA = multiple system atrophy; MSA-C = multiple system atrophy with predominant cerebellar ataxia; REM = rapid eye movement

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Introduction

Recently a progressive neurological syndrome, termed fragile X-associated tremor ataxia syndrome (FXTAS), has been recognized in a subgroup of adult carriers of the *FMR1* (fragile site mental retardation 1 gene) premutation (55–200 repeats). Full mutations in the 5′-untranslated region of this gene (>200 repeats) cause fragile X syndrome, the most common genetic cause of mental retardation, and are usually accompanied by methylation-coupled silencing of the gene. Until recently, premutations had only been associated with premature ovarian failure. Typically, FXTAS patients present with progressive intention tremor and/or gait ataxia, mild parkinsonism and autonomic dysfunction (Hagerman *et al.*, 2001; Berry-Kravis *et al.*, 2003; Jacquemont *et al.*, 2003). Clinical features sometimes also include mild cognitive deficits and peripheral neuropathy. Neuropathological examination of four male and one female FXTAS patients has demonstrated the presence of widespread eosinophilic intranuclear inclusions in both neurons and astrocytes throughout the brain (Greco *et al.*, 2002; Hagerman *et al.*, 2004).

In the general population, an *FMR1* premutation carrier frequency of 1/813 males (95% confidence interval 1/1781 to 1/527) (Dombrowski *et al.*, 2002) and 1/259 females (95% confidence interval 1/373 to 1/198) (Rousseau *et al.*, 1995) has been reported. The disparity in male–female frequency is largely explained by the presence of two X-chromosomes in females and one in males. Smaller CGG repeat expansions (40–54 repeats, grey zone alleles) may expand to a full mutation within two generations (Zhong *et al.*, 1996; Nolin *et al.*, 2003), but the clinical relevance of grey zone alleles has not been demonstrated so far.

Results of previous studies on the prevalence of *FMR1* premutation alleles within populations with movement disorders have not been consistent. In three cohorts of male patients referred for spinocerebellar ataxia testing, significant proportions of premutation carriers were found: three out of 59 patients (5%) (Macpherson *et al.*, 2003), five out of 122 patients (4.1%) (Van Esch *et al.*, 2005) and six out of 275 patients (2.2%) (Brussino *et al.*, 2005), whereas a fourth study (Zuhlke *et al.*, 2004) identified none out of 269 male and only one out of 241 female patients as premutation carrier. In contrast, no premutation carriers were found in three other studies: (i) a screen of 81 patients with essential tremor (Garcia Arocena *et al.*, 2004); (ii) a mixed cohort of 167 patients with essential tremor, sporadic cerebellar ataxia, multiple system atrophy (MSA; $n = 15$ patients) and atypical parkinsonism (Tan *et al.*, 2004); and (iii) 414 patients with clinically diagnosed parkinsonism (Toft *et al.*, 2004). Moreover, two studies analysed the frequency of *FMR1* premutations in MSA patients and did not identify any premutation carrier among 77 (36 male, 41 female) (Yabe *et al.*, 2004) and 65 (40 male, 25 female) patients (Garland *et al.*, 2004).

Based on the current descriptions of FXTAS, MSA appears to be a possible phenocopy and therefore a potentially important differential diagnosis. In particular, both disorders are characterized by a middle- to late-onset combination of cerebellar ataxia, levodopa-unresponsive parkinsonism, and autonomic/urogenital failure. There is considerable uncertainty about the significance of this clinical overlap, especially given the possible genetic implications of FXTAS and the poor prognosis of MSA compared with FXTAS. Moreover, there is a need to test the validity of current MSA diagnostic criteria in the context of FXTAS. To address these questions we determined the *FMR1* CGG repeat length in 507 patients with clinically diagnosed or pathologically proven MSA and 622 healthy controls, and assessed the specificity of the widely used diagnostic consensus criteria for MSA (Gilman *et al.*, 1999) in the light of the recently identified FXTAS.

Patients and methods

Patients and controls

Five hundred and seven MSA patients (253 male, 254 female) were included by collaboration among 12 centres of the European MSA Study Group (EMSA-SG; http://www.emsa-sg.org/). All patients had a clinical diagnosis of MSA by at least one movement disorder specialist. Three groups of patients (possible, probable or pathologically proven), according to established diagnostic consensus criteria (Gilman *et al.*, 1999), were analysed (Table 1). Two hundred and sixty patients were of British origin, 178 German, 22 Austrian, 17 Italian, 13 Danish, 10 Spanish and seven Swedish. This cohort comprised 203 patients with Gilman-possible, 223 patients with Gilman-probable and 81 patients with pathologically proven (definite) MSA (Table 1).

A total of 622 sex- and age-matched healthy individuals (373 male, 249 female) were used as controls for this study. These included 387 individuals from the KORA (Cooperative Health Research in the Region of Augsburg) survey 2000, which is a representative sample of the adult general population of Germany (Illig *et al.*, 2003). The remaining controls ($n = 235$) came from five participating EMSA centres and were primarily of British ethnicity.

FMR1 genotyping

Blood samples were collected from participants after obtaining informed consent, and DNA was extracted using standard procedures. PCR amplification of the *FMR1* CGG repeat was carried out using previously described primers and conditions (Fu *et al.*, 1991). CGG repeat length was determined using an ABI 3100 Avant genetic analyser and Genescan software package (Applied Biosystems). DNA samples from patients with fragile X syndrome (>200 repeats) and premutation carriers, including both small (55–70 repeats) and large (100–200 repeats) premutation alleles, were used as positive controls. For intercentre validation of sizing, test cases representative of different *FMR1* allele size ranges were run at both genotyping sites (Tübingen, London) and allele sizes were compared, confirming accurate allele sizing at both sites. Southern blot analysis (Gasteiger *et al.*, 2003) was performed in premutation carriers to confirm allele sizes and in the majority of apparently homozygous female patients to rule out the presence of possible full mutation or large premutation alleles.

Table 1 Diagnostic criteria of patients and *FMR1* repeat length

Diagnostic criteria	No. of patients	Normal <40 CGG	Grey zone 40–54 CGG	Premutation 55–200 CGG
Possible MSA-C	76	71	5	0
Possible MSA-P	85	76	9	0
Probable MSA-C	76	68	5	3
Probable MSA-P	147	137	9	1
Definite MSA-C	37	36	1	0
Definite MSA-P	44	44	0	0
Total possible MSA*	203	189	14	0
Total probable MSA	223	205	14	4
Total definite MSA	81	80	1	0

*Of 203 patients with possible MSA, 42 could not be subcategorized into either MSA-C or multiple system atrophy with predominant parkinsonian features (MSA-P).

Table 2 Radiological and clinical characteristics of *FMR1* premutation carriers

	Patient 1 (female)	Patient 2 (female)	Patient 3 (male)	Patient 4 (male)
Radiological				
WML MCP	No	No	No	No
WML cerebral	No	No	No	Yes
Atrophy	Yes	Yes	Yes	Yes
Actual finding	Hot-cross bun sign	Slight hyperintense putaminal rim		Asymmetrical WML cerebellum and pons
Clinical				
Tremor (aao)	No	No	At rest/head titubation (68)	At action and posture (50)
Gait ataxia (aao)	Yes (48)	Yes (57)	Yes (58)	Yes (63)
Parkinsonism (aao)	No	Yes (58)	No	Yes (65)
Memory impairment	No	No	Yes	Yes
Executive dysfunction	No	No	No	Yes
Neuropathy	Yes	Unknown	No	Yes
Autonomic failure/urinary symptoms	Yes	Yes	Yes	Yes
CGG repeats	30/63	31/61	111×	71×
MSA diagnosis	Probable MSA-C	Probable MSA-P	Probable MSA-C	Probable MSA-C
Features atypical of MSA	10 years + ambulant, severe disturbance of proprioception	Recurrent falls at disease onset	10 years + ambulant head titubation	10 years + ambulant, orthostatic tremor

WML = white matter lesions; MCP = middle cerebellar peduncles; aao = age at onset; FXTAS = fragile X tremor ataxia syndrome; MSA = multiple system atrophy; MSA-P = multiple system atrophy with predominant parkinsonian features.

Results

Molecular findings

Premutations

Among 223 patients with Gilman-probable MSA, we identified two males and two females with an *FMR1* premutation (1.8%). Within the subgroup of patients with probable MSA with predominant cerebellar ataxia (MSA-C), which is most likely to mimic the phenotype of FXTAS, three of 76 patients (3.95%) carried premutations, which is significantly different from controls ($P < 0.001$). We did not identify any premutation carrier among 81 patients with pathologically proven MSA or 203 patients with possible MSA. In contrast, amongst 622 controls (249 female, 373 males), we identified one female (0.16%) with an allele in the borderline premutation size range (31/55). Clinical characteristics of premutation carriers and *FMR1* repeat lengths are summarized in Table 2.

Grey-zone alleles

The allele distributions for patients and controls are shown in Table 3. We did not observe a statistically significant association between *FMR1* grey-zone alleles (40–54 repeats) and MSA (χ^2 test; $P = 0.15$). The observed frequencies for grey-zone and premutation alleles (Table 1) in our control population were similar to those observed in previous studies and other populations (Crawford *et al.*, 2001).

Clinical findings

Patient 1 presented with ataxia of stance, gait and limbs, which started at 48 years of age. Eleven years after the onset of motor symptoms, this female patient was still ambulatory.

Table 3 Allele frequencies of *FMR1* repeat length in MSA patients and controls

No. of *FMR1* CGG repeats	MSA (n = 761 alleles)		Controls (n = 871 alleles)	
	Male (n = 253)	Female (n = 508)	Male (n = 373)	Female (n = 498)
40–54 (grey zone)	9 (3.56%)	20 (3.94%)	12 (3.22%)	21 (4.22%)
55–200 (premutation)	2 (0.79%)	2 (0.39%)	0	1 (0.20%)

Dysarthria was mild. Pyramidal signs were observed at both upper extremities. Although plantar responses were flexor, brisk tendon reflexes and spasticity in the lower limbs were present. Oculomotor testing revealed downbeat nystagmus, saccadic smooth pursuit and impaired suppression of the vestibulo-ocular reflex. Proprioception and exteroception were severely disturbed. In addition, the patient complained of urge incontinence. There was no tremor or cognitive impairment. Nerve conduction studies yielded evidence of demyelinating and axonal sensorimotor neuropathy, for which no other cause was found. Somatosensory evoked potentials after stimulation of the tibial nerve at the ankle and of the median nerve at the wrist were prolonged. MRI of the brain demonstrated atrophy of the cerebellum, the parietal cortex and the cervical spinal cord and a hot-cross bun sign with increased T2 signal intensity in the basis pontis, but not in the middle cerebellar peduncles. This patient fulfilled diagnostic criteria for both possible FXTAS (Jacquemont *et al.*, 2003) and Gilman-probable MSA-C.

Patient 2 developed micrographia and difficulties in performing fine motor skills, including handwriting, at 57 years of age, followed several months later by progressive gait ataxia with retropulsion and recurrent falls. These symptoms did not respond to high-dose levodopa treatment. She also complained of urinary incontinence. Neurological examination at age 58 years revealed gaze-evoked nystagmus, gait ataxia with retropulsion and an inability to tandem-walk, hypomimia and bradydiadochokinesis, but no rigidity or tremor. Brain MRI showed marked generalized atrophy, especially of the frontal cortex and cerebellar hemispheres, as well as a slight hyperintense rim in the dorsolateral putamen. Striatal D2 dopamine receptor binding of iodobenzamide was found to be reduced bilaterally on SPECT (single photon emission computed tomography) imaging. Bradykinesia, gait ataxia and oculomotor dysfunction progressed rapidly, and the patient died 4 years later at 62 years of age. This patient fulfilled diagnostic criteria for both possible FXTAS and Gilman-probable multiple system atrophy with predominant parkinsonian features (MSA-P).

Patient 3 first presented with mild gait ataxia aged 58 years, which progressed very slowly, and he was still ambulant 10 years after symptom onset. The family history was negative, except for his 86-year-old mother, who was said to be forgetful. At around the same time as he first noticed ataxic symptoms, he became impotent. Subsequently, further autonomic symptoms developed, including nocturia, increased urinary frequency (every 30–45 min), urgency and dribbling incontinence. Urodynamic studies revealed marked detrusor instability and a postmicturition residual volume of 200 ml, necessitating intermittent self-catheterization. He also complained of postural faintness and had several syncopal episodes. Although cognitive function was not formally assessed, his wife was concerned about memory lapses.

At examination at 68 years of age, he was still ambulatory and had not experienced any falls. Neurological examination further revealed normal eye movements, slurring dysarthria and head titubation. In the limbs there was finger–nose and heel–shin ataxia, as well as dysdiadochokinesis. Postural stability was markedly impaired, although he walked on a narrow base. Apart from a mild intermittent rest tremor of the left arm, there was no other clinical evidence of parkinsonism. Tone, power and reflexes were all normal, but the plantar responses were clearly extensor. There was no postural drop in blood pressure. MRI of the brain demonstrated moderately severe generalized atrophy, without signal change.

The clinical diagnosis of MSA-C was made by two consultant neurologists. When the second neurologist (N.P.Q.) made this clinical diagnosis, he nevertheless noted that the patient was atypical because of the benign course and the absence on MRI of infratentorial features associated with MSA-C. In retrospect, the head titubation and rest tremor (in the absence of other parkinsonism features) were also atypical. The history and examination fulfilled diagnostic criteria for both possible FXTAS and Gilman-probable MSA-C.

Patient 4 first noticed an action tremor of his right hand and arm aged about 50 years, which gradually worsened, and spread to involve his left arm and right leg. In the family history, his mother suffered from 'shaking hands'. Two years later he developed symptoms of rapid eye movement (REM) sleep behaviour disorder. Aged 63 years, he developed slurring of speech and gait unsteadiness, which was initially episodic and described as 'vertiginous', but eventually became constant. He also developed hearing problems in his left ear, requiring a hearing aid.

Examination at age 65 years, 15 years after symptom onset, revealed a coarse arm tremor, present on posture and action but completely abolished at rest. In addition, almost immediately on standing, he developed a tremor in his legs with major difficulty on starting to walk. Gait was broad-based and he seemed more comfortable walking than standing. His speech was severely dysarthric, and there was arm dysmetria and rebound as well as heel–shin ataxia. By this time, he had severe jerky arm tremor on posture and at rest (but not classical pill-rolling), and had developed mild akinesia and rigidity. In addition, there was marked postural instability. Eye

movement examination revealed hypometric saccades and saccadic intrusion during pursuit, but a full range of movement. Deep tendon reflexes were normal, but there was a subjective diminution of light touch and pinprick to the ankles.

This man also had clinical evidence of autonomic failure, including impotence from age 58 years and increased urinary frequency and urgency. Postmicturition bladder residual volume was 300 ml. An anal sphincter electromyography was marginally abnormal. Formal cardiovascular autonomic function tests revealed postural hypotension on tilt but not on standing, minimal sinus arrhythmia during deep breathing, and blocked blood pressure responses with the Valsalva manoeuvre.

MRI of the brain revealed moderate atrophy above and severe atrophy below the tentorium. Asymmetrical white matter changes were noted in the cerebrum, cerebellum and pons and were reported as compatible with small-vessel disease. Neuropsychological assessment showed moderate generalized cognitive decline from the patient's premorbid level. Finally, nerve conduction studies were abnormal, with evidence of a mixed sensorimotor axonal peripheral neuropathy, for which no other cause was found on investigation.

A clinical diagnosis of MSA-C was assigned (N.P.Q.). The history, examination, and molecular and radiological findings fulfilled criteria for both probable FXTAS and Gilman-probable MSA-C.

Disease duration and peripheral neuropathy in *FMR1* premutation-negative MSA patients

Three out of four *FMR1* premutation carriers remained ambulant more than 10 years after diagnosis, and two had symptoms of peripheral neuropathy (Table 2), both features that are considered atypical of MSA. Therefore, we determined the frequency of these features in those *FMR1* premutation-negative MSA patients where information was available. Only 35 of 387 patients (9.0%) had disease duration of more than 10 years, and 27 of 188 patients (14.4%) showed clinical features of peripheral neuropathy. These findings are consistent with previous studies on disease progression and survival (Watanabe *et al.*, 2002) and frequency of peripheral neuropathy (Pramstaller *et al.*, 1995) in MSA, confirming both features as infrequent in the majority of our *FMR1* premutation-negative MSA patients.

Discussion

Since the initial observations of potential clinical overlap between patients with MSA and FXTAS, there has been considerable interest in the possibility that the condition of some patients diagnosed with MSA may actually be due to *FMR1* premutations. We report the results of the EMSA-SG screening in a large series of MSA patients for *FMR1* premutations. Out of 507 patients with clinically diagnosed or pathologically proven MSA (253 male, 254 female), we identified four patients with premutation alleles (two male and two female). Although all four patients formally fulfilled Gilman consensus criteria for probable rather than possible MSA, three of the four progressed much more slowly than is usual in MSA: they remained ambulant for 10–15 years after diagnosis, rendering a diagnosis of MSA unlikely.

Although intention tremor has been reported in the majority of patients with probable or definite FXTAS (Berry-Kravis *et al.*, 2003; Jacquemont *et al.*, 2003), in our study only a single patient had an action tremor, one had rest tremor (head titubation) and two patients had no tremor at all. None of our patients had symmetrical white matter lesions involving the middle cerebellar peduncles, which are considered a typical but not necessary characteristic of FXTAS (Brunberg *et al.*, 2002; Jacquemont *et al.*, 2003; Storey and Billimoria, 2005). Two of our patients (patients 1 and 4) had white matter lesions in the pons. Both of these patients also had electrophysiological evidence of polyneuropathy. Thus, only one out of four premutation carriers (patient 4) met the proposed diagnostic criteria for probable FXTAS (Jacquemont *et al.*, 2003). However, these FXTAS diagnostic criteria are based solely on the examination of affected premutation carriers ascertained through families with a known fragile X proband and, with more premutation carriers being identified in screens of populations with movement disorders, may be subject to change in the future.

One out of four *FMR1* premutation carriers (patient 4) showed symptoms of REM sleep behaviour disorder, which, to our knowledge, has not been observed previously in FXTAS patients. Whether this possible association holds true for a larger series of FXTAS patients remains to be determined.

The role of *FMR1* premutations in females is not as clear as it is in males. The proposed pathogenic mechanism, i.e. an RNA toxic gain of function model, predicts that affected females may occur. Recently, a series of five female patients with definite or probable FXTAS has been reported (Hagerman *et al.*, 2004). However, all of these patients had large *FMR1* premutation alleles (between 78 and 93 repeats), and 73 female premutation carriers examined in other previous studies did not show significant symptoms of FXTAS (Berry-Kravis *et al.*, 2003; Jacquemont *et al.*, 2004). In this study, we found two female premutation carriers in the MSA cohort. Both female patients had *FMR1* repeat expansions in the lower size range (61 and 63 repeats). In the controls, we identified one female with a premutation allele in the borderline size range (55 repeats) among 249 healthy female controls, which is in accordance with previously reported prevalence rates (between 1/246 and 1/468) (Crawford *et al.*, 2001), and none amongst 373 male controls. Therefore, although our observed prevalence rate for female *FMR1* premutation carriers among MSA patients (1 in 127 patients) is higher than in controls, we cannot rule out the possibility that our finding of *FMR1* premutation alleles in these two female MSA patients could be coincidental, particularly in patient 2, who died after only 5 years of disease.

In contrast, our finding of two male patients with FXTAS among 253 male MSA patients (0.8%) is unlikely to be

coincidental, given an expected prevalence for probable FXTAS of ~1/3000 for males aged >50 years in the general population, and a disease progression much slower than typically seen in MSA (Hagerman and Hagerman, 2004). In conclusion, our data suggest that Gilman-probable MSA is only rarely associated with *FMR1* premutations. This is reassuring with regard to the specificity of current MSA diagnostic criteria. Therefore, routine *FMR1* genotyping in typical MSA patients, in the absence of a positive family history of fragile X mental retardation syndrome, cannot be recommended. However, in probable MSA patients with unusually slow disease progression, predominant tremor and/or peripheral neuropathy, FXTAS should be considered, especially in patients with probable MSA-C, a subgroup in which we found a significantly elevated frequency of premutation carriers (3.95%) compared with controls (0.16%).

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