

Abnormal Nerve Conduction Features in Fragile X Premutation Carriers

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Background: Distal neuropathy is part of the clinical phenotype in most males with the fragile X–associated tremor/ataxia syndrome (FXTAS) caused by the 55 to 200 CGG repeat expansion.

Methods: We performed nerve conduction studies in 16 male carriers with FXTAS, 11 non-FXTAS carriers, and 11 control subjects and assessed the outcomes with respect to the fragile X mental retardation 1 genotype (*FMRI*) (Online Mendelian Inheritance in Man [OMIM] 309550; NT011681) and messenger RNA expression.

Results: Men with FXTAS had slower tibial nerve conduction velocities and prolonged F-wave latencies compared with controls ($z=2.06$, $P=.04$; and $z=2.73$, $P=.005$) and unaffected premutation males ($z=1.98$, $P=.04$; and $z=2.00$, $P=.04$). Compound muscle action potential amplitudes were smaller in the FXTAS group relative to controls. Sural nerve action potential amplitudes were re-

duced in the FXTAS group compared with controls. After controlling for age, there was a significant relationship between the longer CGG repeat number and tibial nerve conduction velocity slowing ($r=-0.42$, $P=.04$) and between elevated messenger RNA levels and reduction of the tibial compound muscle action potential velocity ($r=-0.52$, $P=.01$) in the premutation group.

Conclusions: Male premutation carriers had significant conduction abnormalities of motor and sensory nerves that correlated with molecular measures, suggesting that the premutation *FMRI* genotype is a causal factor. There was also evidence of nerve conduction abnormalities in non-FXTAS carriers compared with controls, which suggests that the neuropathy can occur without the full clinical presentation of FXTAS.

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FRAGILE X–ASSOCIATED tremor/ataxia syndrome (FXTAS) is a neurodegenerative syndrome that occurs in approximately 38% of carriers of premutation expansions (55-200 CGG repeats; noncoding) of the fragile X mental retardation 1 gene (*FMRI*)¹⁻⁴ who have been ascertained through families with fragile X syndrome probands. Premutation CGG repeat expansions are associated with a 2- to 8-fold elevation in *FMRI* messenger RNA (mRNA) levels,^{5,6} resulting in mRNA gain-of-function toxic effects and formation of intranuclear inclusions in neurons and astrocytes of carriers with FXTAS.⁷⁻⁹ Analysis of the FXTAS-associated inclusions has identified several of the constituent proteins, including lamin A/C and other neurofilaments, myelin basic protein, α B-crystallin, and hnRNPA2.⁹ There is evi-

dence of active dysregulation of lamin A/C in neural cells after transfection with the premutation-sized CGG repeats.

The core features of FXTAS are intention tremor, ataxia, or both, which usually begin after age 50 years (mean age at onset, 62 years), with an increase in incidence and prevalence with age.^{1,10} Enhanced white matter intensities (T2-weighted magnetic resonance images) in the middle cerebral peduncles, a major radiologic feature of FXTAS, are found in approximately 60% of affected men.³ Additional features include parkinsonism, cognitive decline beginning with executive function deficits and memory problems, autonomic dysfunction, and neuropathy.^{2,4} The present study evaluates nerve conduction in male carriers with and without FXTAS compared with controls and the relationship of *FMRI* molecular measures to the nerve conduction study (NCS).

Table 1. Demographic Characteristics of the 38 Study Participants

Variable	Control Subjects (n=11)	Premutation Carriers	
		With FXTAS (n=16)	Without FXTAS (n=11)
Age, mean (SD), y	61.00 (8.45)	64.69 (7.66)	66.36 (5.59)
<i>FMR1</i> molecular analyses, mean (SD) ^a			
CGG repeat length	29.40 (2.27)	103.13 (16.36)	85.00 (15.36)
<i>FMR1</i> messenger RNA levels (times novel)	1.39 (0.10)	3.34 (0.75)	2.85 (0.2)
Ethnicity, No. (%)			
White	8 (73)	15 (94)	10 (91)
Hispanic	2 (18)	0	1 (9)
Other	1 (9)	1 (6)	0

Abbreviation: FXTAS, fragile X-associated tremor/ataxia syndrome.
^aMeasured in CGG repeat length.

METHODS

PARTICIPANTS

Thirty-eight men participated in the study, including 16 carriers with FXTAS (mean [SD] age, 64.69 [7.66] years), 11 carriers without FXTAS (mean [SD] age, 66.39 [5.59] years), and 11 controls without the premutation (mean [SD] age, 61.00 [8.45] years). In this prospective study, patients with FXTAS were ascertained through the Fragile X Research and Treatment Center, M.I.N.D. Institute, University of California, Davis, Sacramento, among families with fragile X-related disorders. They were enrolled in this study consecutively as they were diagnosed as having FXTAS. They were not ascertained by the presence of neuropathy. The electromyography clinician was masked to the status of the patient and the molecular studies. The 3 groups did not differ significantly in age ($F_{2,37}=1.54$, $P=.23$). All the patients signed informed consent forms approved by the University of California, Davis, institutional review board to undergo this protocol.

All the participants received a detailed clinical history and a careful medical and complete neurologic examination as previously described elsewhere.^{10,11} The diagnosis of definite or probable FXTAS was confirmed according to the criteria previously reported by Jacquemont et al,³ including the presence of tremor, ataxia, or both combined with magnetic resonance imaging findings documenting white matter lesions and generalized brain atrophy. Almost all of the patients with FXTAS had a lack of reflexes at the ankles and decreased or absent vibration sense in the feet.

NERVE CONDUCTION STUDIES

A certified electromyography technician (G.F.-F.) in the electrodiagnostic laboratory of the University of California, Davis Medical Center performed all the NCSs following guidelines provided by the American Association of Neuromuscular and Electrodiagnostic Medicine. During the electrodiagnostic evaluation, the limb temperature was maintained at 33°C. An electromyographic system (Viking IV; Nicolet Instrument Corp, Madison, Wisconsin) was used for all NCSs. We evaluated motor and sensory nerve function in the right posterior tibial and right sural nerves.

For the tibial NCSs, the compound muscle action potential (CMAP) was recorded using an active surface electrode placed

over the belly of the abductor hallucis muscle while the tibial nerve was supramaximally stimulated distally behind the medial malleolus at the ankle and proximally at the popliteal fossa. The tibial F-wave response was elicited via supramaximal stimulation of the tibial nerve at the ankle. For the sural NCSs, the sensory nerve action potential (SNAP) was recorded using an active surface electrode placed behind the lateral malleolus and with antidromic nerve stimulus delivered slightly distal to the lower border of the belly of the lateral gastrocnemius muscle at a median distance of 130 mm proximal to the active recording electrode.

We assessed 5 variables and defined the measurement methods for each as follows:

1. The CMAP amplitude was measured (in millivolts) from peak to peak.
2. Conduction velocity (CV) of CMAP was calculated (in meters per second) by dividing the distance or length (in millimeters) between the ankle and the popliteal fossa by the latency difference between proximal and distal stimulation.
3. Temporal dispersion was defined by the presence or absence of change in the configuration of the M-wave response resulting from proximal nerve stimulation and the ratio obtained by dividing the duration of the M-wave response resulting from distal nerve stimulation by that resulting from proximal nerve stimulation.
4. F-wave latency was defined as the time elapsed from initial tibial nerve stimulation to the starting point of the F wave (the shortest latency of a minimum of 10 F waves was used as the measuring variable).
5. The SNAP amplitude was measured (in millivolts) from the first positive peak to the highest negative peak.

MOLECULAR STUDIES

In all the patients, the length of the CGG repeat number was analyzed by means of Southern blot and polymerase chain reaction analysis, and the level of *FMR1* mRNA was documented using methods previously described elsewhere.⁵

STATISTICAL ANALYSIS

Analyses were performed using a statistical software program (SPSS for Windows Version 14.0; SPSS Inc, Chicago, Illinois). The distribution of data for each variable in each group was graphically displayed and reviewed for normality and variance homogeneity. Outlier values in each variable and skewness in 2 of the variables necessitated nonparametric analyses of group differences using the Kruskal-Wallis test and correlations with the molecular data using the Spearman rho. If any variable indicated a significant difference, then post hoc Mann-Whitney tests were used to specify the group differences.

RESULTS

The results of NCS variables of the posterior tibial and sural nerves are displayed in **Tables 1, 2, 3,** and **4.** Analyses were separated into male premutation carriers with and without FXTAS compared with their controls. Kruskal-Wallis tests with group (control vs premutation with FXTAS vs premutation without FXTAS) as the independent variable and 5 nerve conduction variables (CMAP amplitude, CV of CMAP, SNAP amplitude, F-wave latency, and presence of abnormal temporal dispersion) as the dependent variables revealed significant group differences in CMAP amplitude, F-wave latency, and SNAP amplitude. The group effect on CV of CMAP approached significance.

Results of post hoc Mann-Whitney tests showed that compared with controls, men with FXTAS demonstrated significantly reduced CMAP amplitude, slower CV of CMAP, reduced SNAP amplitude, and prolonged F-wave latency. Patients with FXTAS also had slower CV of CMAP ($z=1.98, P=.04$) and more prolonged F-wave latencies ($z=2.00, P=.04$) relative to premutation carriers

Table 2. Abnormal Nerve Conduction Variables by Study Group

Variable	Control Subjects, No. (%) (n=11)	Premutation Carriers, No. (%)	
		With FXTAS (n=16)	Without FXTAS (n=11)
Abnormalities in ≥ 1 variable	1 (9)	14 (88)	4 (36)
Reduced CMAP amplitude	0	6 (38)	1 (9)
Slow CV of CMAP	0	5 (31)	1 (9)
Change in M-wave configuration (temporal dispersion)	1 (9)	12 (75)	4 (36)
Prolonged F-wave latency	0	9 (56)	3 (27)
Unobtainable SNAP amplitude	0	3 (19)	0

Abbreviations: CMAP, compound muscle action potential; CV, conduction velocity; FXTAS, fragile X-associated tremor/ataxia syndrome; SNAP, sensory nerve action potential.

Table 3. Nerve Conduction Measures for Controls vs Male Carriers With or Without FXTAS

Variable	Control Subjects, Mean (SD)	Premutation Carriers, Mean (SD)	
		With FXTAS	Without FXTAS
CMAP amplitude, mV	16.91 (6.16)	10.11 (6.22)	13.17 (7.97)
CV of CMAP, m/s	44.73 (4.22)	41.19 (7.02)	44.45 (2.91)
Duration ratio of the M wave (temporal dispersion)	1.15 (0.29)	1.31 (0.18)	1.18 (1.04)
F-wave latency, ms	52.03 (5.99)	60.33 (6.87)	54.48 (7.24)
SNAP amplitude, mV	10.64 (3.56)	7.23 (2.65)	7.64 (3.04)

Abbreviations: CMAP, compound muscle action potential; CV, conduction velocity; FXTAS, fragile X-associated tremor/ataxia syndrome; SNAP, sensory nerve action potential.

without FXTAS. Finally, compared with controls, premutation carriers without FXTAS had lower SNAP amplitude, but this difference only approached significance.

Spearman ρ correlations between CGG repeat size and *FMRI* mRNA level with each of the NCS variables revealed that a higher CGG repeat size was associated with slower CV of CMAP in the combined sample of men with the premutation ($\rho=-0.47, P=.01$). The more elevated mRNA levels were associated with either slower CV of CMAP ($\rho=-0.38, P=.05$) or more prolonged F-wave latency ($\rho=-0.40, P=.05$). Age was correlated with CMAP amplitude ($\rho=-0.59, P=.02$) and CV of CMAP ($\rho=-0.47, P=.07$) in the FXTAS group. Age was not significantly correlated with any other variables in any of the 3 groups. To ensure that the significant association between CGG repeat size and CV of CMAP was not confounded by age, a partial correlation between these variables covarying for age was computed, and this correlation remained ($r=-0.42, P=.04$). The analogous partial correlation between mRNA and CMAP velocity covarying for age in the premutation group was strengthened ($r=-0.52, P<.01$).

COMMENT

Male fragile X premutation carriers with FXTAS exhibited multiple aberrations of motor and sensory nerve conduction variables compared with controls and non-FXTAS male carriers. Compared with controls, males with FXTAS demonstrated significantly reduced tibial CMAP and sural SNAP amplitudes, slow CV of CMAP, and prolonged F-wave latency. Motor CV was slow, and F-wave latency was also significantly prolonged in FXTAS vs non-FXTAS carriers. Only the FXTAS group had evidence of demyelination, with significant slowing of motor CV and prolongation of F-wave latencies compared with non-FXTAS carriers and controls. In addition, there was a significant reduction in CMAP and SNAP amplitudes in the FXTAS group compared with controls and non-FXTAS carriers, which also supports an axonal disease in patients with FXTAS. Seventy-five percent of male carriers with FXTAS had abnormalities in temporal dispersion, and only 2 of 16 men (13%) with FXTAS had normal variables on all studies. In the broader context of FXTAS, these data demonstrate that neuropathy is part of the neurologic phenotype, as has been demonstrated in case stud-

Table 4. Between-Group Analysis of Nerve Conduction Variables

Variable	Control Subjects vs FXTAS Carriers		Control Subjects vs Non-FXTAS Carriers		FXTAS vs Non-FXTAS Carriers	
	z Score	P Value	z Score	P Value	z Score	P Value
Reduced CMAP amplitude	2.64	.007 ^a	1.42	.16	0.49	.62
Slow CV of CMAP	2.06	.04 ^a	0.30	.76	1.98	.04 ^a
Presence of temporal dispersion		.04 ^a	0.95	.34	1.32	.19
Prolonged F-wave latencies	2.73	.005 ^a	0.95	.34	2.00	.04 ^a
Reduced SNAP amplitude	2.72	.006 ^a	1.92	.06	0.41	.68

Abbreviations: CMAP, compound muscle action potential; CV, conduction velocity; FXTAS, fragile X-associated tremor/ataxia syndrome; NS, not significant; SNAP, sensory nerve action potential.

^aStatistically significant ($P<.05$).

ies^{12,13} but not by quantitative measures before the present article.

The correlations of slowed motor CV with increasing CGG repeat length, and of slower motor CV and more prolonged F-wave latencies with increasing mRNA levels, suggest that these nerve conduction changes are related to the premutation (greater RNA toxic effects associated with larger CGG repeat size and elevated mRNA levels). These molecular correlations have been seen with FXTAS central nervous system neuropathologic changes on magnetic resonance images (including brain atrophy and white matter disease).¹⁴ The present data demonstrate that these molecular clinical correlations are also associated with peripheral neurologic disease. The pathologic mechanism of RNA toxicity leading to neuropathic changes in patients with FXTAS is hypothesized to involve lamin A/C dysregulation, which occurs in patients with FXTAS.¹⁵ Lamin A/C dysregulation due to specific mutations in the lamin A/C gene (*LMNA*) (Online Mendelian Inheritance in Man [OMIM] *150330) has been shown to be responsible for at least 1 type of peripheral neuropathy, Charcot-Marie-Tooth type 2B1.¹⁶ Therefore, FXTAS may reflect an underlying functional laminopathy related to the deleterious effects of elevated levels of expanded CGG repeat mRNA in premutation carriers.

Several non-FXTAS carriers had temporal dispersion (36%) and prolonged F-wave latency (27%). These nerve conduction abnormalities may be prodromal of the ensuing neurologic signs of tremor and ataxia in FXTAS. Further longitudinal studies are needed to validate this assumption. The FXTAS may present with neuropathy before the onset of tremor and ataxia.¹³ It is, therefore, recommended that patients with neuropathy and a family history of fragile X or cognitive deficits, premature ovarian failure (also related to the premutation), or autism (related to the full mutation) be tested for the fragile X premutation.

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