

Neuropathic Features in Fragile X Premutation Carriers

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurological condition occurring in fragile X premutation carriers, predominantly males, and resulting in CNS dysfunction including tremor, ataxia, Parkinsonism, and cognitive decline. Neuropathic signs have also been described. The objective of this study was to compare neuropathic signs in fragile X premutation carriers versus controls and determine the relationship of these signs to CGG repeat length and tremor/ataxia. A neuropathy scale was utilized to compare distal tendon reflexes and vibration sense in subjects from a large cohort of carriers and controls undergoing neurological exam and structured videotaping sessions for movement disorder rating. The male carrier group displayed more impairment on total neuropathy, vibration and reflex scores than the corresponding control

group, while female carriers were not significantly different from controls. In males, after correction for age effects, there was a correlation between CGG repeat length and both total neuropathy and reflex impairments. Age-adjusted partial correlation analyses showed an association between neuropathy scores and severity of ataxia but not tremor in carrier males and females. These data suggest that neuropathic signs are associated with the fragile X premutation, presumably occurring through the same mechanism proposed for CNS disease, namely, toxicity from expanded-CGG-repeat *FMR1* mRNA. © 2006 Wiley-Liss, Inc.

Key words: fragile X syndrome; FXTAS; neuropathy; ataxia; *FMR1*; FMRP

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INTRODUCTION

Trinucleotide (CGG) repeat expansion mutations in the promoter region of the fragile X mental retardation 1 (*FMR1*) gene result in fragile X syndrome (FXS) when a full mutation (>200 CGG repeats) is present [Verkerk et al., 1991]. The fragile X-associated tremor/ataxia syndrome (FXTAS) is associated with smaller expansions in the premutation range (55–200 repeats) [Hagerman et al., 2001; Berry-Kravis et al., 2003; Jacquemont et al., 2003, 2004a; Hagerman and Hagerman, 2004]. The full mutation results in FXS through methylation and transcriptional silencing of *FMR1* [Pieretti et al., 1991] with consequent absence or deficiency of *FMR1* protein (FMRP) [Devys et al., 1993]. Although the premutation predisposes an individual to have

children or grandchildren with FXS, it is not associated with hypermethylation of *FMR1*, transcriptional silencing, absence of FMRP, nor typical FXS [Fu et al., 1991; Devys et al., 1993]. Individuals with the premutation may display some of the physical features of FXS [Hagerman, 1996], and behavioral features such as anxiety, obsessive compulsive behavior, and social phobia, are more prevalent in

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premutation carriers than in control groups [Dorn et al., 1994; Hessler et al., 2005].

A subgroup of older (>50 years) predominantly male premutation carriers develop fragile X-associated tremor/ataxia syndrome (FXTAS), a progressive neurological condition characterized predominantly by multidimensional complex tremor, gait and limb ataxia, and parkinsonian symptoms [Hagerman et al., 2001; Berry-Kravis et al., 2003; Jacquemont et al., 2003, 2004a; Hagerman and Hagerman, 2004]. MRI findings in affected individuals include diffuse brain atrophy with evidence of white matter disease and characteristic high signal lesions in the middle cerebellar peduncles [Brunberg et al., 2002]. Associated neuropathological findings include eosinophilic intranuclear inclusions in neurons and astroglia, and spongiform change in the white matter with mild axonal and myelin loss [Greco et al., 2002, 2006]. FXTAS is thought to result from cellular toxicity related to the expanded CGG repeat-containing *FMRI* mRNA, a mechanism that is entirely distinct from that operating in FXS [Hagerman and Hagerman, 2004]. Consistent with the RNA toxicity model, FXTAS is not seen in individuals with FXS and a full mutation, as the *FMRI* mRNA is generally reduced or absent in these individuals due to transcriptional silencing.

FXTAS affects predominantly male premutation carriers [Berry-Kravis et al., 2003; Jacquemont et al., 2004a] although female carriers do occasionally have the clinical and neuropathologic features of FXTAS [Hagerman et al., 2004; Zuhlke et al., 2004; Berry-Kravis et al., 2005; Jacquemont et al., 2005]. The neurological symptoms are generally milder and less progressive in females than in males, presumably due to a variable degree of protection provided by the expression of *FMRI* from the normal X chromosome in a percentage of cells [Berry-Kravis et al., 2005; Jacquemont et al., 2005].

In addition to tremor and ataxia, a variety of features have been reported in some subjects with FXTAS, including neuropathy, autonomic dysfunction, psychiatric and executive function deficits, and progressive cognitive deterioration [Hagerman et al., 2001; Jacquemont et al., 2003, 2004a]. No studies, however, have systematically addressed the question of whether signs of peripheral neuropathy are associated with the premutation carrier state, with CGG repeat number, or with other features of FXTAS. In this pilot study, we sought to determine whether clinical findings suggestive of neuropathy are found more frequently in premutation carriers, as compared to an age-matched control population.

METHODS

Subjects

All subjects were participants in a multicenter study (University of California, Davis, School of Medicine,

University of Colorado at Denver Health Sciences Center, and RUSH University Medical Center in Chicago) to characterize neurological findings in premutation carriers, over a time period extending from 2002 to 2005. Participants were ascertained through a proband with fragile X syndrome, and were eligible to participate if they were over 50 years of age and were parents, maternal grandparents, or great aunts/uncles of the proband. Great aunts/uncles were included if either husband/wife was an obligate carrier of a *FMRI* premutation based on pedigree analysis. In this instance, prior knowledge of carrier status was not required for participation, since the presumed non-carrier spouse generally participated as a control. Participation was offered to all families presenting in clinic with a fragile X proband regardless of medical or neurological history. To minimize bias, the study was presented as an information gathering effort to characterize neurological and psychological symptoms that might be associated with the premutation carrier state. All eligible family members were encouraged to participate regardless of symptoms and carrier status. No specific emphasis was placed on recruitment of individuals with tremor, ataxia, or other neurological signs. All participants signed informed consent. The study was approved by Institutional Review Boards at RUSH University Medical Center, University of California at Davis School of Medicine, and University of Colorado at Denver Health Sciences Center.

Neuropathy Screening Scale

As part of the study protocol, all enrolled subjects had a neurological examination. The initial focus of the study was the movement disorder typically associated with FXTAS. Therefore the examination of the peripheral nervous system was insufficiently detailed to utilize currently validated neuropathy scales used for quantifying neuropathic findings in hereditary motor and sensory neuropathies (HSMN) [Krajewski et al., 2000]. Data was available for >90% of subjects for all sites for reflexes and vibration testing in the lower extremities. Data was felt to be valid as reflexes and vibration were tested in a standardized fashion by a neurologist or developmental pediatrician well versed in the use of maneuvers such as passive ankle dorsiflexion or fist clenching to bring out apparently absent reflexes. Reflexes were recorded in the charts as per standard notation for the neurological examination: clonus, increased, normal, decreased, or absent. Vibration was recorded as percent of sternal reference. A scale to screen for peripheral neuropathy in the cohort using the available neurological exam data for ankle reflexes and vibratory sensation in the feet was defined as follows. For the ankle reflex impairment score, a zero was given for each the right and left

ankle for a normal or increased reflex, a one for a decreased reflex, and a two for an absent reflex. For the vibratory loss score, a zero was given for normal or mild loss (75%–100% of sternal reference), a one for moderate loss (10%–75%), and a two for severe loss (absent or <10%). Thus, a subject would achieve a maximum (total) impairment score of 8 (4 for reflexes, 4 for vibration) if there were full reflex and vibratory loss bilaterally in the distal lower extremities. Likewise, a subject would receive a score of zero for completely normal function without evidence of neuropathy. Total score and individual scores for reflexes and vibration were entered into a computerized database for analysis.

FXTAS Scale

Subjects underwent a structured videotaping session, which was modified from a previously described protocol [Berry-Kravis et al., 2003] and was designed to capture the major motor features of FXTAS: tremor, cerebellar dysfunction, and parkinsonism. Videotapes were scored by movement disorder neurologists (CG, ML, LZ) blinded to the subjects' premutation status, utilizing the FXTAS Rating Scale, which was developed by our group for the multi-center study and refined through use in over 200 persons. The FXTAS Rating Scale is a combination of the Clinical Rating Scale for Tremor (CRST) [Fahn et al., 1998], items addressing handwriting, pouring, and drawing, most items from the International Cooperative Ataxia Rating Scale (ICARS) [Trouillas et al., 1997], all items from the Unified Parkinson's Disease Rating Scale (UPDRS) [Fahn et al., 1987], and the tandem test from the Unified Huntington's Disease Rating Scale [Huntington Study Group, 1996]. Good to excellent (weighted kappa >0.4) interrater reliability was established for the rating neurologists on all items from an independent rating of 5% of randomly selected videotapes. Sub-domains measuring each motor feature of interest were designated for tremor, cerebellar dysfunction, and parkinsonism. The FXTAS Rating Scale total score ranges from 0 to 226. The tremor sub-domain (maximum score 53) assesses action and postural tremor, including assessment of drawing and handwriting. The ataxia sub-domain (maximum score 73) assesses posture and gait, limb ataxia, dysarthria, and oculomotor disturbances. The parkinsonism sub-domain (maximum score 100) assesses bradykinesia, gait and balance, rest tremor and rigidity.

Molecular Analyses

FMR1 DNA analysis was performed according to a PCR assay described previously [Levinson et al., 1994], which provides specific allele sizes (number of

CGG repeats) throughout the normal and premutation ranges. Normal (<45 CGG repeats), intermediate (also referred to as "gray zone") (45–54 repeats), and premutation (55–200 repeats) alleles are as defined by Maddalena et al. [2001]. For the purpose of the current analysis, we have incorporated somewhat smaller alleles into the intermediate range (41–54 CGG repeats) to account for varying usage in the literature as well as the intrinsic uncertainty in the definition of that range. The broadening of the intermediate range has no effect on the conclusions of this study.

The *FMR1* locus was subsequently analyzed for all premutation carriers by Southern blot with probe StB12.3, following *Eco* RI/*Eag* I digestion [Rousseau et al., 1991]. This second approach served to rule out mosaicism for a full mutation (>200 repeats) and/or partial gene methylation (which would not be detected in the PCR assay), genetic lesions that might be expected to differ from the premutation in terms of clinical effects. DNA from females with apparent homozygosity for a single allele in the PCR assay was also analyzed by Southern blot to rule out presence of a full mutation or very large premutation allele, which may not be amplified in the PCR reaction.

Statistical Analyses

Comparison of the total and sub-domain neuropathy scores between the premutation and control groups was accomplished utilizing the two-sample *t*-test. Comparisons were performed for male and female subject groups separately. The association between the total and individual sub-domain neuropathy scores and CGG repeat length was analyzed by linear regression, with neuropathy scores as dependent variables, and CGG and age as independent variables. To quantify the association between FXTAS scale scores (overall and subscores) and neuropathy scores (total and component scores) in premutation carriers, partial correlation coefficients were computed to adjust for the effect of age. Statistical analyses were performed using the software packages SAS [SAS Institute Inc, 2000–2004] and R [R Development Core Team, 2005].

In view of the uncertainty regarding the potential for involvement in carriers of intermediate alleles, coupled with variable definitions of the range itself (e.g., 41–54 vs. 45–54; see above) [Hagerman and Hagerman, 2004], seven cases with CGG repeats that fall within the broader range (41–54 CGG repeats) were eliminated from the group-wise comparisons. Individuals with those alleles are included in the regression analyses performed across the full range of allele sizes (include both controls and carriers) but are excluded from the regression involving premutation carriers only and from the correlation analyses between FXTAS and neuropathy scores.

RESULTS

A total of 233 subjects were enrolled in the study during the 2002–2005 time period. Neurological exam data were available for 207 subjects, including 99 males and 108 females. DNA analyses subdivided groups into 49 premutation carrier males, 46 non-carrier control males, 73 premutation carrier females, and 32 non-carrier control females. There were four males and three females with intermediate alleles. Age was similar in all four groups and was not significantly different between the carrier and control groups for either sex: mean \pm SD = 66.9 ± 8.3 for male carriers, 64.3 ± 9.69 for male non-carriers ($P=0.16$ for males), 63.0 ± 9.9 for female carriers, and 64.8 ± 9.8 for female non-carriers ($P=0.32$ for females). The premutation allele size distribution was typical of that seen in populations of premutation carriers derived from FXS families [Jaquemont et al., 2004b], with the majority of alleles falling in the 70–100 repeat range (for males mean \pm SD for CGG repeat number was 91.3 ± 19.1 , range 60–147; for females 83.2 ± 16.4 , range 56–150). Mean allele size and distribution of normal alleles was similar to that described in other cohorts [Brown et al., 1996].

The male premutation carrier group had significantly higher mean scores on the total neuropathy screening scale ($P=0.0014$), the vibration score alone ($P=0.015$), and the reflex score alone ($P=0.0014$), than the male control group, indicating more impairment of both distal vibratory sense and reflexes in the premutation carrier males (Table I). There were significantly fewer males in the premutation carrier group with a completely normal score of zero, while there were many more premutation carriers with very high scores of 7–8 (Fig. 1). By contrast, there was no significant difference between female carriers and controls for total score, vibration score, or reflex score (Table I).

Regression analyses across the entire CGG repeat range (normal to premutation) showed correlations between age and total, vibration, and reflex scores in combined carrier and non-carrier males, and

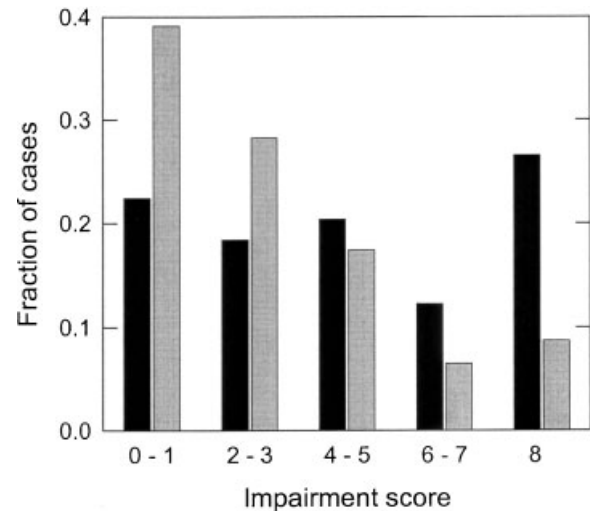


FIG. 1. Relative distribution of total neuropathy scores in the male control and premutation carrier populations, plotted as the fraction of the total number of premutation carriers (black bars) and controls (gray bars) having a total neuropathy score in the specified range. A score of zero represents a completely normal exam and a score of 8 represents the most severe deficit (absence of vibration sense and ankle reflexes bilaterally). The graph demonstrates clearly the increased proportion of premutation carriers with scores in higher ranges and decreased proportion of carriers with scores in the lower ranges.

between age and total and vibration scores in combined carrier and non-carrier females (Table II), with the result of more severe impairment with increasing age. In males, after correction for age, there was a highly significant correlation between CGG repeat length and total neuropathy score ($P=0.0005$) and reflex score ($P=0.00007$), such that increasing CGG repeat length was associated with more severe impairment (Table II). There was also a trend toward a correlation between CGG repeat length and vibration score ($P=0.06$). In females, after age correction, there was also a correlation between CGG repeat length and total neuropathy score ($P=0.04$) and trends for vibration ($P=0.12$) and reflex ($P=0.08$) scores (Table II).

TABLE I. Group Comparisons for Neuropathy Scores in Premutation Carriers and Controls

	Control		Premutation		P^b
	Mean	SD ^a	Mean	SD ^a	
Males					
Total neuropathy score	2.46	2.56	4.18	2.93	0.0014
Vibration score	1.22	1.49	1.92	1.63	0.016
Reflex score	1.24	1.46	2.27	1.78	0.0014
Females					
Total neuropathy score	2.06	2.29	2.55	2.46	0.17
Vibration score	1.12	1.36	1.3	1.3	0.27
Reflex score	0.94	1.52	1.25	1.63	0.18

^aSD, standard deviation.

^bThese comparisons were not corrected for age since there was no significant difference in age of carrier and control groups for either gender.

TABLE II. Regression Analyses With Age and Mutation Repeat Length

	Age ^a	CGG repeat length ^a
Males		
Total neuropathy score	0.0003	0.0005
Vibration score	0.0019	0.063
Reflex score	0.0021	0.00007
Females		
Total neuropathy score	0.0014	0.041
Vibration score	0.00005	0.12
Reflex score	0.16	0.076
Males with premutation only		
Total neuropathy score	0.012	0.0037
Vibration score	0.21	0.42
Reflex score	0.0015	0.00002

^aValues presented are P -values for linear regression. Regressions of scores against CGG repeat lengths were controlled for age.

When the association between CGG repeat length and neuropathy scores was analyzed with age correction (Table II) in the group of premutation males alone, the total score was correlated with repeat size ($P=0.0037$). This effect was predominantly driven by the reflex score, which showed a strong correlation ($P=0.00002$), while vibration score did not correlate with repeat size ($P=0.42$). Further, in the male premutation group, total and reflex scores were correlated with age, but vibration score was not (Table II).

Data from both the neuropathy screening scale and the FXTAS rating scale were available for 49 male carriers and 73 female carriers. There was no association between total neuropathy, reflex, or vibratory scores and FXTAS scale tremor subscore for either male or female carrier groups. However, age-adjusted partial correlation analyses (Table III) demonstrated significant correlations between the total neuropathy score and the ataxia subscore (males $P=0.018$, females $P=0.045$) but not the combined tremor/ataxia subscore or overall FXTAS scale score. The reflex score was also significantly correlated with the ataxia subscore in both males ($P=0.0115$) and females ($P=0.0192$), and with the combined tremor/ataxia subscore in males only ($P=0.0474$), but not the overall FXTAS scale score. Clearly, the correlation between the overall neuropathy score and the ataxia subscore was driven by the reflex score, as no association was seen between vibration score and ataxia subscore, combined tremor/ataxia subscore, or overall FXTAS scale score.

DISCUSSION

Although there have been prior descriptions of peripheral neuropathy in *FMRI* premutation carriers with FXTAS [Hagerman et al., 2001; Jacquemont et al., 2003, 2004a,b], the current report represents the first demonstration that signs of neuropathy on clinical examination are associated with premutation carrier status. Both loss of distal reflexes and

reduction in vibratory sense were observed in premutation carrier males. In addition, this report identifies a strong correlation between CGG repeat length and total neuropathy score in males. This correlation was driven predominantly by the reflex score, suggesting that the finding of loss of reflexes is more dependent on mutation length than vibratory loss. Both reflex and vibratory loss were correlated with age, but vibratory loss was more dependent on age than CGG repeat length in carrier males. Reduced reflexes and vibratory sense are common findings in aging populations due to problems like spinal disease, arthritis, and dependent edema, and certainly were seen to some extent in the control group. However, despite similarity in age, the findings are significantly amplified in the male premutation carrier group and the relationship with mutation length, even when the male premutation carrier group was analyzed alone, suggests that these signs of neuropathy are specifically related to the fragile X premutation and the degree of clinical involvement is a function of the size of the CGG repeat expansion. No significant group differences were seen between female carriers and controls, likely due to decreased penetrance of the symptoms related to the presence of a normal *FMRI* allele. The absence of a significant difference in the female groups presumably reflects the broad variation in clinical involvement among carriers as a result of variation in activation ratio; nonetheless, we cannot rule out other sex-specific effects on phenotype. There was, however, a correlation between CGG repeat length and total neuropathy score in females, and a trend toward correlation of reflex and vibration scores with mutation length. This is consistent with the hypothesis that a pattern of deficits similar to that observed in males exists in females, but is partially obscured by X-inactivation effects. It is expected that the X-inactivation ratio, which defines the relative expression of the normal and premutation alleles, impacts the prevalence and degree of severity of symptoms in females [Berry-Kravis et al., 2005; Jacquemont et al., 2005]. Correction for X-inactivation ratio might allow

TABLE III. Partial Correlation Analyses for Neuropathy and Fxtas Scores

	Video FXTAS score		
	Ataxia ^a	Tremor + Ataxia ^a	Overall ^{a,b}
Males with premutation (N = 49)			
Total neuropathy score	0.339 (0.018)	0.221 (0.114)	0.221 (0.131)
Vibration score	0.214 (0.145)	0.101 (0.491)	0.115 (0.438)
Reflex score	0.362 (0.012)	0.288 (0.047)	0.259 (0.075)
Females with premutation (N = 72)			
Total neuropathy score	0.237 (0.045)	0.083 (0.488)	0.190 (0.109)
Vibration score	0.103 (0.389)	0.001 (0.997)	0.101 (0.401)
Reflex score	0.276 (0.019)	0.124 (0.299)	0.208 (0.080)

^aValues presented are partial correlation, adjusted for age, and corresponding P -values are given in parenthesis, with significant P -values shown in bold.

^bOverall video FXTAS score is tremor + ataxia + Parkinsonism.

an association between neuropathic findings and premutation carrier status to be more readily observed.

The exam deficits identified in this pilot study are most consistent with a large fiber sensory neuropathy. Exam data for this cohort is not sufficiently detailed to determine whether there is also an increased incidence of pain/temperature loss relevant to small unmyelinated sensory fibers, or whether there exist subtle motor strength deficits. Although motor weakness has not been an obvious or frequent feature in carrier subjects examined through this study, motor strength was not systematically addressed in this protocol and there exist some reports of muscle weakness in previous case studies of FXTAS [Jacquemont et al., 2003, 2004b]. The clinical findings presented here should now prompt a more comprehensive assessment of a carrier and control cohort using including EMG/NCV to confirm presence and type of neuropathy.

Identification of the correlation between the FXTAS scale ataxia subscore and the total neuropathy score and reflex subscore in premutation carriers provides a direct tie between a core feature of FXTAS and neuropathy. Further, despite the lack of significant group differences between female carriers and controls, the presence of ataxia and neuropathy occurred together in female carriers, as activation ratios exert an equal effect on both variables in this analysis (i.e., if a female has an unfavorable X-chromosomal activation ratio, leading to clinical manifestations of ataxia, she is also likely to develop the peripheral component of the disease). This observation provides support to the concept of a similar pattern of FXTAS involvement in females as males, with lower frequency of manifestations, and also strongly supports the hypothesis that neuropathy occurs as a part of the overall manifestations of FXTAS. The correlation with ataxia but not with tremor could be interpreted as occurring because ataxia is the most significant feature of FXTAS. However, tremor and ataxia are both core features of FXTAS, [Jacquemont et al., 2003, 2004a,b; Berry-Kravis et al., 2003], and tremor is more common than ataxia as the presenting feature of FXTAS [Leehey et al., 2004]. An alternative interpretation for the correlation between ataxia and peripheral neuropathy is that part of the measured ataxia from the FXTAS ataxia subscale is explained by peripheral sensory nerve dysfunction; while the tremor and parkinsonian subscales are measuring purely central disease.

It is thought that FXTAS occurs through an RNA gain-of-function mechanism in which accumulated mRNA containing the expanded CGG repeat exerts a toxic effect by sequestering and perturbing function of nuclear proteins [Greco et al., 2002, 2006; Hagerman and Hagerman, 2004]. This mechanism is consistent with the findings of nuclear

inclusions containing *FMR1* mRNA in brains from individuals with FXTAS [Tassone et al., 2004] and mice expressing a *dFmr1* gene with a premutation expansion allele (~100 CGG repeats) [Van Dam et al., 2005], and with studies showing neurodegeneration in *Drosophila* expressing an expanded CGG repeat [Jin et al., 2003].

More recent data show that lamin A/C is sequestered within the intranuclear inclusions [Arocena et al., 2005; Iwahashi et al., 2006] and is dysregulated in a neural cell transfection system upon expression of the expanded CGG repeat [Iwahashi et al., 2006]. Lamin A/C are isoforms of the product of the *LMNA* gene (OMIM *150330); they are nuclear intermediate filament proteins [Worman and Courvalin, 2004] that are involved in diverse nuclear functions, including maintenance of the nuclear membrane and heterochromatin structure, particularly in response to mechanical stress. Mutations in *LMNA* cause a number of diverse diseases, depending on the location of the mutation. Interestingly, one of the *LMNA* mutations gives rise to an autosomal recessive axonal neuropathy, Charcot-Marie-Tooth type 2B1 (OMIM *605588). Thus, it is possible that the peripheral neuropathy associated with premutation alleles and FXTAS reflects a functional laminopathy [Arocena et al., 2005]. In this regard, the initial findings on MRI of individuals with FXTAS involve abnormal signal in white matter tracts, suggesting there may be an axonopathy common to both CNS and peripheral nerve, which develops as a result of CGG repeat-containing mRNA-mediated toxicity.

A weakness of this study is the lack of sufficient data to fully characterize and quantify neuropathy using a standardized neuropathy scale. Future detailed studies of peripheral nervous system disease in carriers of the fragile X premutation should include systematic quantification of motor and sensory neuropathic signs on a standardized scale and quantitative motor and sensory testing in a protocol similar to that validated for HSMN studies [Krajewski et al., 2000] as well as electrodiagnostic studies in both males and females. Clinical analyses aimed at evaluating whether ataxia in FXTAS is derived from central or peripheral nervous system effects or both will be crucial to help us understand whether there are separate factors predisposing premutation carriers to peripheral and/or central disease.

This report demonstrates that clinical signs of neuropathy are a previously understudied aspect of the phenotype associated with the fragile X premutation and demonstrates a relationship between clinical severity and mutation size and an association between severity of neuropathy and ataxia. Expansion and characterization of the fragile X premutation-associated phenotype continues to provide new insights into the spectrum of disease that may be seen in association with nervous system toxicity mediated by repeat-containing mRNA.

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REFERENCES

- Arocena DG, Iwahashi CK, Won N, Beilina A, Ludwig AL, Tassone F, Schwartz PH, Hagerman PJ. 2005. Induction of inclusion formation and disruption of lamin A/C structure by premutation CGG-repeat RNA in human cultured neural cells. *Hum Mol Genet* 14:3661–3671.
- Berry-Kravis E, Lewin F, Wu J, Leehey M, Hagerman R, Hagerman P, Goetz CG. 2003. Tremor and limb ataxia in fragile X premutation carriers: Blinded videotape evaluation. *Ann Neurol* 53:616–623.
- Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG. 2005. Penetrance of fragile X-associated tremor/ataxia syndrome (FXTAS) in two sisters related to X-inactivation pattern. *Ann Neurol* 57:144–147.
- Brown WT, Nolin S, Houck G, Jr, Ding X, Glicksman A, Li SY, Stark-Houck S, Brophy P, Duncan C, Dobkin C, Jenkins E. 1996. Prenatal diagnosis and carrier screening for fragile X by PCR. *Am J Med Genet* 64:191–195.
- Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, Tassone F, Brown WT, Greco CM, Hagerman PJ. 2002. Fragile X premutation carriers: Characteristic MR imaging findings in adult males with progressive cerebellar and cognitive dysfunction. *Am J Neuroradiology* 23:1757–1766.
- Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL. 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nature Genetics* 4:335–340.
- Dorn MB, Mazzocco MMM, Hagerman RJ. 1994. Behavioral and psychiatric disorders in adult male carriers of fragile X. *J Am Acad Child Adolesc Psychiatry* 33:256–264.
- Fahn S, Elton RL, and members of the UPDRS Development Committee. 1987. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne DB, Godstein M, editors. *Recent developments in Parkinson's Disease*. Florham Park, NJ: Macmillan Health Care Information. p 153–164.
- Fahn S, Tolosa E, Marin C. 1998. Clinical rating scale for tremor. In: Jankovic J, Tolosa E, editors. *Parkinson's disease and movement disorders*. Baltimore-Munich: Urban & Schwarzenberg. p 225–234.
- Fu Y-H, Kohl DPA, Pizzuti A, Pieretti M, Sutcliffe J, Richards S, Verkerk AJMH, Holden JJA, Fenwick RG, Jr, Warren ST, Oostra BA, Nelson DL, Caskey CT. 1991. Variation of the CGG repeat at the fragile X site results in genetic instability and resolution of the Sherman paradox. *Cell* 67:1047–1058.
- Greco C, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ. 2002. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain* 125:1760–1771.
- Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, Trapp BD, Iwahashi C, Brunberg J, Grigsby J, Hessler D, Becker EJ, Papazian J, Leehey MA, Hagerman RJ, Hagerman PJ. 2006. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). *Brain* 129:243–255.
- Hagerman RJ. 1996. Physical and behavioral phenotype. In: Hagerman RJ, Cronister A, editors. *Fragile X syndrome: Diagnosis, treatment and research*. Baltimore: Johns Hopkins University Press. p 3–87.
- Hagerman PJ, Hagerman RJ. 2004. The fragile X premutation: A maturing perspective. *Am J Hum Genet* 74:805–816.
- Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. 2001. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 57:127–130.
- Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessler D, Harris SW, Zhang L, Jardini T, Gane LW, Ferranti J, Ruiz L, Leehey MA, Grigsby J, Hagerman PJ. 2004. Fragile X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 mutation. *Am J Hum Genet* 74:1051–1056.
- Hessler D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, Barbato I, Rice C, Gould E, Hall DA, Grigsby J, Wegelin J, Harris S, Lewin F, Weinberg D, Hagerman PJ, Hagerman RJ. 2005. Abnormal elevation of FMR1 mRNA is associated with psychopathology in adults with the fragile X premutation. *Am J Med Genet Part B Neuropsychiatr Genet* 139B:115–121.
- Huntington Study Group. 1996. Unified Huntington's disease rating scale: Reliability and consistency. *Mov Disord* 11:136–142.
- Iwahashi CK, Yasui DH, An HJ, Greco CM, Tassone F, Nannan K, Babineau B, Lebrilla CB, Hagerman RJ, Hagerman PJ. 2006. Protein composition of the intranuclear inclusions of FXTAS. *Brain* 129:256–271.
- Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ. 2003. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. *Am J Hum Genet* 72:869–878.
- Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, Zhang L, Jardini T, Gane LW, Harris SW, Herman K, Grigsby J, Greco C, Berry-Kravis E, Tassone F, Hagerman PJ. 2004a. Penetrance of the fragile X-associated tremor/ataxia syndrome (FXTAS) in a premutation carrier population: Initial results from the California-based study. *JAMA* 291:460–469.
- Jacquemont S, Orrico A, Galli L, Sahota PK, Brunberg JA, Anichini C, Leehey M, Schaeffer S, Hagerman RJ, Hagerman PJ, Tassone F. 2005. Spastic paraparesis, cerebellar ataxia, and intention tremor: A severe variant of FXTAS? *J Med Genet* 42:e14.
- Jacquemont S, Farzin F, Hall D, Leehey M, Tassone F, Gane L, Zhang L, Grigsby J, Jardini T, Lewin F, Berry-Kravis E, Hagerman PJ, Hagerman RJ. 2004b. Aging in individuals with the FMR1 mutation. *Am J Mental Retardation* 109:154–164.
- Jin P, Zarnescu D, Zhang F, Pearson CE, Lucchesi JC, Moses K, Warren ST. 2003. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in *Drosophila*. *Neuron* 39:739–747.
- Krajewski KM, Lewis RA, Fuerst DR, Turansky C, Hinderer SR, Garber J, Kamholz J, Shy ME. 2000. Neurological dysfunction and axonal degeneration in Charcot-Marie-Tooth disease type 1A. *Brain* 123:1516–1527.
- Leehey MA, Hall DA, Rice CD, Jacquemont S, Zhang L, Grigsby J, Hagerman RJ, Hagerman PJ, Berry-Kravis E, Leehey MA. 2004. The clinical course of fragile X-associated tremor/ataxia syndrome (FXTAS). *Mov Disord* 19:112.
- Levinson G, Maddalena A, Palmer FT, Harton GL, Bick DP, Howard-Peebles PN, Black SH, Schulman JD. 1994. Improved sizing of fragile X CCG repeats by nested polymerase chain reaction. *Am J Med Genet* 51:527–534.
- Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. 2001. Technical standards and guidelines for fragile X: The first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med* 3:200–205.

- Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, Nelson DL. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 66:817–822.
- RDevelopment Core Team. 2005. R: A language and environment for statistical computing. Version 2.2. Vienna, Austria.
- Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boue J, Tommerup N, Van Der Hagen C, DeLozier-Blanchet C, Croquette M-F, Gilgenkrantz S, Jalbert P, Voelckel M-A, Oberle I, Mandel J-L. 1991. Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. *N Engl J Med* 325:1673–1681.
- SAS Institute Inc. 2000–2004. SAS. Version 9.1. Cary, NC.
- Tassone F, Iwahashi C, Hagerman PJ. 2004. *FMR1* RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). *RNA Biology* 1:103–105.
- Trouillas P, Takayanagi T, Hallet M, Currier RD, Subramony SH, Wessel F, Bryer A, Diener HC, Massaquoi S, Gomez CM, Coutinho P, Ben-Hamida M, Campanella G, Filla A, Schut L, Timann D, Honnorat J, Nighoghossian N, Manyam B. 1997. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. *J Neuro Sci* 145:205–211.
- Van Dam D, Errijgers V, Kooy RF, Willemsen R, Mientjes E, Oostra BA, De Deyn PP. 2005. Cognitive decline, neuromotor and behavioural disturbances in a mouse model for fragile-X-associated tremor/ataxia syndrome (FXTAS). *Behav Brain Res* 162:233–239.
- Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang F, Eussen BE, van Ommen G-JB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST. 1991. Identification of a gene (FMR-1) containing a CGG repeat co-incident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–914.
- Worman HJ, Courvalin J-C. 2004. How do mutations in lamins A and C cause disease? *J Clin Invest* 113:349–351.
- Zuhlke C, Budnik A, Gehlken U, Dalski A, Purmann S, Naumann M, Schmidt M, Burk K, Schwinger E. 2004. FMR1 premutation as a rare cause of late onset ataxia. *J Neurol* 251:1418–1419.