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Reduced Telomere Length in older Men with Premutation Alleles of the Fragile X Mental Retardation 1 Gene

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Abstract

Reduced telomere length has recently been reported in T lymphocytes of individuals with trisomy 21 Down syndrome (DS) and dementia. Shorter telomeres also have been documented in dyskeratosis congenita, cell senescence, Alzheimer disease, and neoplastic transformation. These observations suggest that similar shortening may occur in people with fragile X-associated tremor/ataxia syndrome (FXTAS), which frequently is accompanied by dementia. To test this hypothesis, telomere length has been quantified in T lymphocytes from older male carriers of premutation *FMRI* alleles, with or without FXTAS, and FXTAS with dementia. Shorter telomeres (relative to age-matched controls) were observed in 5/5 individuals with FXTAS and dementia, in 2/2 individuals with FXTAS without dementia, and in 3/3 individuals with the fragile X premutation only (p values ranged from <.001 to <.05; Student's *t* test), indicating that telomere shortening is associated with the premutation expansion of the *FMRI* gene. The current study design allowed simultaneous comparisons among control, premutation, FXTAS, and FXTAS with dementia samples, and showed nearly equal degrees of shortening relative to controls among the three premutation sample groups. Thus, telomere shortening may serve as a biomarker for cellular dysregulation that may precede the development of the symptoms of FXTAS.

Keywords

telomere shortening; metaphase; FMR1; Parkinson; dementia; FXTAS

INTRODUCTION

There is general agreement that telomere shortening occurs with increasing age [Hastie et al., 1990; Lindsey et al., 1991]. Moreover, there is growing evidence that telomere shortening may play a causative role in certain age-related human disorders, including heart disease [Samani et al., 2001; Benetos et al., 2004], osteoporosis [Valdes et al., 2007], obesity [Valdes et al., 2005], dyskeratosis congenita [Vulliamy and Dokal, 2007], Alzheimer disease [Panossian et al., 2003], and Down syndrome [Jenkins et al., 2006a]. The recent discovery of UUAGGG-repeat telomeric RNAs may help to better understand the role of the telomere in the above conditions including aging and cancer [Schoeftner and Blasco, 2007].

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We have recently reported shorter telomeres in T lymphocytes of people with trisomy 21 (Down syndrome; DS) with dementia relative to DS without dementia [Jenkins et al., 2006a]. As an extension of these studies, we have investigated whether the same association between telomere shortening and dementia occurs in some cases of the late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS) [Hagerman and Hagerman, 2007; Jacquemont et al., 2007; Bourgeois et al., 2007; Berry-Kravis et al., 2007]. FXTAS, which affects carriers (mainly males) of premutation alleles (55-200 CGG repeats) of the fragile X mental retardation 1 gene (*FMR1*), is often, but not invariably, accompanied by dementia [Grigsby et al., 2006]. Therefore, the principal objective of the current study was to test the hypothesis that T lymphocytes from males with FXTAS and dementia will exhibit shorter telomeres than either FXTAS cases without dementia, or carriers without FXTAS or age-matched controls.

MATERIALS AND METHODS

Subjects

Subjects who were carriers of premutation alleles, documented by *FMR1* genotyping, were recruited through the Fragile X Research and Treatment Center at the University of California at Davis MIND Institute. Subjects signed informed consent for this research, which was reviewed and approved by an Institutional Review Board (IRB). Each subject underwent detailed neurological studies to confirm the presence of FXTAS, and neuropsychological testing to evaluate the presence of cognitive deficits. Using the diagnostic criteria for FXTAS reported by Jacquemont et al. [2003] and the diagnostic criteria for dementia of DSM-IV [Ghaemi et al., 2008], patients were separated into 3 groups: 5 with FXTAS and dementia; 2 with FXTAS but without dementia and 3 with the premutation and without FXTAS (see Table I).

Molecular Analysis

Genomic DNA was isolated from peripheral blood leucocytes (5 ml of whole blood) using standard methods (Puregene Kit; Gentra Inc.). Southern blot analysis and PCR analysis were performed as described by Tassone et al. [2008]. Analysis and calculation of the repeat size were carried out using an Alpha Innotech FluorChem 8800 Image Detection System.

Telomere length analysis

Anonymous frozen buffy coat cells were obtained from older (53-73) age-matched, male individuals who: (1) did not carry the fragile X premutation; (2) carried the premutation and were unaffected by FXTAS; (3) had FXTAS, but without dementia; (4) had FXTAS with dementia. The buffy coat samples were cultured at 37°C for four days at an initial concentration of 200,000 – 400,000 viable mononuclear cells per ml of PHA-containing medium.

Telomere length differences were determined by detecting changes in fluorescence intensity using an FITC-labeled peptide nucleic acid (PNA) probe and DAPI counterstaining, (Applied Biosystems; DAKO) and Applied Imaging software similar to that previously reported [Jenkins et al., 2006a] including studies that validated the use of fluorescence intensity measurements for both whole metaphase analyses and individual chromosome comparisons. Nothing similar is possible with Southern analysis [Landsorp et al., 1996; Londoño-Vallejo et al., 2001; Perner et al., 2003; Mayer et al., 2006]. Analyses of 20 metaphase and 20 interphase preparations from short-term T lymphocyte cultures from each of 15 individuals were analyzed using quantitative PNA FISH technology blind to dementia, FXTAS, and premutation status. Pairwise comparisons of light intensity values, e.g., 20 light

intensity values from a fragile X premutation versus 20 light intensity values from an age-matched control, were made using the Student's *t* test.

RESULTS

An example of telomere labeling is depicted in Figure 1, which shows telomeres at the ends of the short and long arms of metaphase chromosomes. Interphase labeling of telomeres is also shown following short-term lymphocyte culture of buffy coat samples from a person with FXTAS and dementia. Table II provides quantitative measures of the mean light intensities detected from metaphases of 15 male individuals with and without the fragile X premutation, FXTAS, and FXTAS with dementia in 7 studies where an age-matched control sample was cultured and analyzed in parallel with a premutation and/or FXTAS and/or FXTAS with dementia sample. Interphase results, uninformative for Study 1, were otherwise similar to those from metaphase (data not shown but is available upon request). As shown in Table II, metaphase analyses showed shorter telomeres in 3 of 3 cases of premutation only, compared to age-matched controls. Similarly, shorter telomeres were observed in 2 of 2 cases of FXTAS compared to age-matched controls, and 5 of 5 cases of FXTAS with dementia exhibited shorter telomeres than age-matched controls. When values for premutation without FXTAS, FXTAS, and FXTAS with dementia were compared in Study 1, there were no statistically significant pairwise differences of light intensities, similar to results for FXTAS, and FXTAS with dementia in Study 2.

The same result was obtained when individual chromosome 1 was analyzed for telomere shortening among the samples listed in Table I. That is, all samples with fragile X premutations with and without FXTAS and/or dementia exhibited shorter telomeres on chromosome 1 than age-matched controls, while this was not observed when individual chromosomes 21 and X were analyzed (data not shown but available upon request).

DISCUSSION

As listed in Table II, 7 studies have clearly shown that telomeres were shorter in 10 older male individuals with the Fragile X premutation with and without FXTAS and/or dementia, compared to age-matched controls. Also, it appears that either whole metaphase analysis or individual chromosome 1 analysis may be used to detect shorter telomeres in older males with the Fragile X premutation. Individual chromosomes were studied as well as whole metaphases and interphases because Zou et al. [2004] have studied whether a subset of short telomeres determines replicative senescence, and because a previous study showed that individual chromosomes 21 had shorter telomeres in people with Down syndrome and dementia versus those with DS only [Jenkins et al., 2006b]. Future studies will show whether other individual chromosomes may be utilized to detect increased telomere shortening in people with the fragile X premutation.

In Studies 1 and 2 (Table II), it was surprising to find virtually no difference in telomere length (based on light intensity differences) from premutation specimens with and without FXTAS and/or dementia. It is possible that increased telomere shortening had already begun in males with the premutation only, as shown here in three of three individuals studied. To confirm the above results, further studies are warranted. Specifically, analysis of the premutation only will be carried out to determine at what age significant telomere shortening begins compared to controls. As can be seen from Table II, premutation lengths did not appear to be inversely correlated with telomere length except for all controls versus all premutations with or without FXTAS and/or dementia. It should also be mentioned that control sample 285-04 had a light intensity value of 216 in Study 1 and of 172 in Study 7, likely due to inter-study variability since all studies could not be done simultaneously.

Our observations of telomere shortening in individuals with the FMR1 premutation remain to be confirmed and elucidated. Additional studies, conducted on younger premutation carriers, will determine whether increased telomere shortening can serve as a biomarker that may result in earlier detection of individuals at increased risk for developing FXTAS and FXTAS/dementia. Early detection could lead to future intervention strategies to extend the quality of life of both them and their families and may even lead to interventions aimed at slowing down or reversing the disease progression.

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Fig. 1.
Telomeres in metaphase and interphase nuclei shown by an FITC-labeled PNA probe with DAPI counterstaining, from a person with FXTAS and dementia

Table 1

CGG repeat number, age, diagnostic and dementia status of 15 older men with and without the fragile X premutation

Log Number	Category	CGG Repeats	Age	FXTAS	Dementia
19-04	normal	32	69	no	no
151-04	normal	33	66	no	no
160-04	normal	33	59	no	no
285-04	normal	30	73	no	no
1313-06	normal	30	64	no	no
384-04	premutation	73	73	no	no
501-05	premutation	60	56	no	no
293-06	premutation	81	69	no	no
176-05	premutation	90	70	yes	no
464-05	premutation	103	65	yes	no
4-04	premutation	87	68	yes	yes
5-04	premutation	88	66	yes	yes
112-04	premutation	90	69	yes	yes
192-04	premutation	115	64	yes	yes
383-05	premutation	142	64	yes	Yes

Shorter telomeres shown by reduced light intensities ($\times 10^3$) in fragile Xpremutations with and without FXTAS and/or dementia in whole metaphases from 7 studies

Table II

Metaphase	Control	Premutation	FXTAS	FXTAS/D	P
Study 1	216 ₇₃ ¹ [285-04] 30cgg	174 ₇₃ [384-04] 73cgg	182 ₇₀ [176-05] 90cgg	182 ₆₉ [112-04] 90cgg	<.003 ²
2	203 ₆₉ [19-04] 32cgg	NA	153 ₆₅ [464-05] 103cgg	137 ₆₆ [5-04] 88cgg	<.000004
3	228 ₆₄ [1313-06] 30cgg	NA	NA	164 ₆₄ [383-05] 142cgg	<.000002
4	181 ₆₆ [151-04] 33cgg	NA	NA	152 ₆₄ [192-04] 115cgg	<.05
5	255 ₆₉ ³ [19-04] 32cgg	NA	NA	169 ₆₈ [4-04] 87cgg	<.000001
6	243 ₅₉ [160-04] 33cgg	147 ₅₆ [501-05] 60cgg	NA	NA	<.00007
7	172 ₇₃ ³ [285-04] 30cgg	93.3 ₆₉ [293-06] 81cgg	NA	NA	<.000001

¹ 21673: means light intensity value = 216 from a 73-year-old control subject whose egg repeat size was 30 and whose log number is [285-04].

² p values are the largest numerical values obtained among comparisons between control vs. premutation; control vs. premutation with FXTAS; control vs. premutation with FXTAS and dementia, within Studies 1-7.

³ different samples from the same control individuals were cultured at different times for Studies 1 and 7 and Studies 2 and 5 so that the total number of control individuals in this project was 5 with a total of 7 cultured control buffy coat samples.