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 \( FMR1 \) 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 \(< 0.001\) to \(< 0.05\); Student’s \( t \)-test), indicating that telomere shortening is associated with the premutation expansion of the \( FMR1 \) 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.

**Key words:** 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 (DS) [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].

We have recently reported shorter telomeres in T lymphocytes of people with trisomy 21 (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 syn-
drome (FXTAS) [Berry-Kravis et al., 2007; Bourgeois et al., 2007; Hagerman and Hagerman, 2007; Jacqueumont 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 three groups: five with FXTAS and dementia; two with FXTAS but without dementia, and three with the premutation and without FXTAS (see Table I).

**Molecular Analysis**

Genomic DNA was isolated from peripheral blood leukocytes (5 ml of whole blood) using standard methods (Puregene Kit; Gentra, Inc., Plymouth, MN). Southern blot 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 4 days at an initial concentration of 200,000–400,000 viable mononuclear cells per milliliter 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, Foster City, CA; DAKO, Carpinteria, CA) 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 [Lansdorp et al., 1996; London˜o-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, for example, 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 seven 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 three of three cases of premutation only, compared to age-matched controls. Similarly, shorter telomeres were observed in two of two cases of FXTAS compared to age-matched controls, and five of five 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, seven studies have clearly shown that telomeres were shorter in 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 DS 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 interstudy 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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