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Telomere Longitudinal Shortening as a Biomarker for Dementia Status of Adults With Down Syndrome

Edmund C. Jenkins,[1]* Lingling Ye,[1] Sharon J. Krinsky-McHale,[1] Warren B. Zigman,[1] Nicole Schupf,[1,2] and Wayne P. Silverman[3]

[1]New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York

[2]Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, New York

[3]The Kennedy Krieger Institute and The Johns Hopkins University School of Medicine, Baltimore, Maryland

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Previous studies have suggested that Alzheimer's disease (AD) causes an accelerated shortening of telomeres, the ends of chromosomes consisting of highly conserved TTAGGG repeats that, because of unidirectional 5′–3′ DNA synthesis, lose end point material with each cell division. Our own previous work suggested that telomere length of T-lymphocytes might be a remarkably accurate biomarker for "mild cognitive impairment" in adults with Down syndrome (MCI-DS), a population at dramatically high risk for AD. To verify that the progression of cognitive and functional losses due to AD produced this observed telomere shortening, we have now examined sequential changes in telomere length in five individuals with Down syndrome (3F, 2M) as they transitioned from preclinical AD to MCI-DS (N = 4) or dementia (N = 1). As in our previous studies, we used PNA (peptide nucleic acid) probes for telomeres and the chromosome 2 centromere (as an "internal standard" expected to be unaffected by aging or dementia status), with samples from the same individuals now collected prior to and following development of MCI-DS or dementia. Consistent shortening of telomere length was observed over time. Further comparisons with our previous cross-sectional findings indicated that telomere lengths prior to clinical decline were similar to those of other adults with Down syndrome (DS) who have not experienced clinical decline while telomere lengths following transition to MCI-DS or dementia in the current study were comparable to those of other adults with DS who have developed MCI-DS or dementia. Taken together, findings indicate that telomere length has significant promise as a biomarker of clinical progression of AD for adults with DS, and further longitudinal studies of a larger sample of individuals with DS are clearly warranted to validate these findings and determine if and how factors affecting AD risk also influence these measures of telomere length. © 2015 Wiley Periodicals, Inc.

Key words: Down syndrome; mild cognitive impairment (MCI); dementia status; biomarker; telomere longitudinal shortening; FISH with PNA (peptide nucleic acid) probes; telomere length in light intensity units; telomere length in microns

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INTRODUCTION

Trisomy 21 [Down syndrome (DS)] is the most prevalent chromosomal cause of intellectual disability (ID), with an incidence rate of approximately one in every 690–730 newborns [Presson et al., 2013]. It is caused by the presence of a third copy of chromosome 21, either the whole chromosome 21 (approximately 97% of cases), as a partial trisomy, or as mosaicism. In previous generations, survival of newborns with DS into middle age was unusual [Presson et al., 2013; Zigman, 2013]. However, survival has increased dramatically since the 1950s, and the population of "older" adults with DS has expanded rapidly as life expectancy has increased to

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*Correspondence to:
Edmund Jenkins, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314.
E-mail: edjenkins.jenkins85@gmail.com
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approach 60 years of age. In fact, some adults with DS now survive into their 70s and 80s [Zigman, 2013].

Aging processes among adults with DS have been of interest for over 100 years because of the high risk of Alzheimer's disease (AD) in this population [Fraser et al., 1876; Jervis, 1948; Zigman et al., 2002; Zigman, 2013]. The formation of β-amyloid (Aβ) plaques in brain has been observed even early in development [Leverenz and Raskind, 1998], and increases markedly in middle age and beyond. Virtually all adults with DS over 40 years of age undergoing autopsy have exhibited key neuropathological characteristics of AD, including deposition of Aβ in diffuse and neuritic plaques and neurofibrillary pathology [Malamud, 1972; Wisniewski et al., 1985; Zigman, 2013]. While the neuropathological manifestations of AD in DS have been attributed, at least in part, to the triplication and over-expression of the gene for amyloid precursor protein located on chromosome 21 [Goldgaber et al., 1987; Tanzi et al., 1987; Rumble et al., 1989), other chromosome 21 genes may also be involved [Wegiel et al., 2011].

Clinically, AD in adults with DS is characterized by mid- to late-life onset of a progressive deterioration of cognition and functional abilities, with considerable variability in behavioral manifestation [Zigman, 2013]. Standard diagnostic methods used to evaluate individuals with suspected dementia in the typically developing population ordinarily are not appropriate for use with adults with DS, many of whom have never developed the specific cognitive and adaptive skills that are measured by these assessment instruments [Krinsky-McHale and Silverman, 2013]. AD is a slowly progressing disease, with a prolonged prodromal period followed by the development of mild cognitive impairment (MCI) [Albert et al., 2011] prior to frank dementia. Recognition and diagnosis of early decline in adults with DS is particularly difficult due to their pre-existing cognitive impairments [Krinsky-McHale and Silverman, 2013]. A biomarker that can confirm a diagnosis of AD in adults with DS during its early stages would be of enormous value as effective treatments, once available, would be most effective before devastating and irreversible damage to the neural substrate has occurred. Further, negative findings with a truly informative biomarker would suggest that observed declines have a cause(s) other than AD and some may be responsive to treatment (e.g., sensory impairments; undiagnosed pain).

Telomeres, highly conserved TTAGGG repeats on the ends of chromosomes, become shorter with each subsequent cell cycle, eventually leading to the inability to replicate [Watson, 1972; Olovnikov, 1973; Montpetit et al., 2014]. Reduced telomere length has been associated with replicative cellular senescence and apoptosis [Allsopp et al., 1992; Hao et al., 2004], tumorigenesis [Plentz et al., 2004], in vivo cellular aging [Flanary and Streit, 2003], heart disease [Haycock et al., 2014], stress [Epel et al., 2004; Puterman et al., 2015], dyskeratosis congenita [Keeling et al., 2014], and AD [Panossian et al., 2003], as well as a host of psychosocial, behavioral, and environmental factors [Starkweather et al., 2014]. There has been considerable interest in telomere length as a biomarker associated with the development of MCI or AD within the typically developing population, and while the preponderance of evidence indicates a relationship [e.g., Mathur et al., 2013], results have been mixed. For example, Movérare-Skrtic et al. [2012] did not find a strong relationship between telomere length and presence of AD, while Honig et al. [2012] found an association, but only in women. In a recent review, Cai et al. [2013] noted that effects could be cell-type-specific and emphasized that additional research is needed to clarify the mechanisms contributing to a relationship between telomere length and AD. It is also interesting to note that Gruszecka et al. [2015] observed, for the first time, that juveniles with DS had significantly longer telomeres than did healthy age-matched controls from the non-affected population. On the other hand, another study revealed shorter telomeres in babies with DS versus controls [Wenger et al., 2014], while it has also been demonstrated that trisomy 21 significantly increases the aging of blood and brain tissue by 6.6 years [Horvath et al., 2015].

For adults with DS, we have reported preliminary findings indicating a highly significant relationship between telomere length in T lymphocytes and the presence of MCI (as operationally defined and subsequently referred to as MCI-DS [Krinsky-McHale and Silverman, 2013; Silverman et al., 2013] and dementia [Jenkins et al., 2006, 2008, 2010, 2012b], paralleling results of Panossian et al. [2003]). These findings, demonstrating perfect sensitivity (i.e., 1.0) and specificity (i.e., 1.0) in detecting MCI-DS and dementia, provide a strong foundation for larger studies.

While our earlier findings demonstrated a strong cross-sectional association between telomere length and clinical dementia status, those results did not provide a direct indication of whether telomere length represents a risk factor, with affected individuals having shorter telomeres prior to MCI-DS/dementia onset, or a biomarker of dementia status, with increased shortening as AD progresses. Prospective longitudinal data were needed to determine if telomeres become shorter with declining clinical status within individuals. We now report that to be the case in five of five adults with DS who transitioned from "clinically normal aging" to either MCI-DS or dementia.

MATERIALS AND METHODS

Human Subjects and Blood Samples

General methods followed those described in our earlier reports [Jenkins et al., 2010, 2012b], with all procedures reviewed and approved by our institutional IRBs. Briefly, a large sample of participants with DS over 45 years of age is being followed prospectively and assessed at baseline and 18 (±4) month intervals thereafter [Silverman et al., 2004]. Assessments include direct cognitive testing, covering domains likely to be affected by the development of AD from its preclinical stage through end-stage dementia. Comprehensive interviews are conducted with knowledgeable informants regarding participants' adaptive functioning, cognitive abilities, health status, and neuropsychiatric/behavioral concerns. Medical chart reviews are accompanied, for participants or their representatives providing consent/assent, by a blood draw of approximately 50 ml, collected via routine phlebotomy for studies of genotype (including karyotype for participants who were not yet tested for trisomy 21), AD risk factors and blood-based biomarkers. Any excess sample is archived for future use.

Following each assessment cycle, all findings were reviewed in a consensus case conference to determine clinical dementia status, classified as "Aging typically/Not demented" (indicating with

reasonable certainty that significant age-associated impairment was absent, "MCI-DS" indicating that there was some indication of mild cognitive and/or functional decline but of a severity insufficient to merit a diagnosis of dementia, or "Possible Definite Dementia" indicating that signs and symptoms of dementia were present. (A small number of cases were classified as uncertain or indeterminate due to the presence of assessment difficulties or the presence of significant complications involving concerns unrelated to AD, but that was not the case for any of the five individuals included in the present study).

The present sample included five adults with DS selected based on the availability of assessment data and blood samples at two points in time, where participants at Time 1 were classified as "aging typically/not demented" while at Time 2 had developed either MCI-DS (N = 4) or Dementia (N = 1). Mean time in years between assessments was 2.94 (SD = 1.81). Selected characteristics of these individuals are provided in Table I. All individuals had full trisomy 21. Prior to telomere studies, samples were coded to avoid any influence of experimenter expectation on results.

For the current study, up to 10 ml of whole blood was collected at each sampling time in a green-topped tube (containing sodium heparin and delivered to our laboratory at room temperature on the same day). Samples were coded using a unique participant ID, along with the date and general demographics. Samples were processed via Ficoll-Paque gradient centrifugation and frozen in liquid nitrogen for future studies. Initial short-term cultures contained 200,000–400,000 mononuclear white blood cells per ml.

Short-term cultured T-lymphocytes from each participant at each of the two study time points were hybridized with an FITC-labeled peptide nucleic acid (PNA) probe (DAKO, North America) as previously described to label telomeres [Lansdorp et al., 1996; Londono-Vallejo et al., 2001; Jenkins et al., 2006, 2008, 2010]. In addition, a centromere 2 (cen 2) PNA probe (a gift for investigational use from DAKO, Glostrup, Denmark) was used to facilitate identification of that chromosome. One to two slides were made from each sample and then hybridized with the PNA probes and counterstained. Twenty metaphases were then chosen at random, digitized, and uploaded onto the MetaSystems image analyzer (ISIS software program) for actual linear measurements of telomere and inter-telomere chromosome lengths. From these measures, ratios were calculated for each cell examined, with telomere length as the numerator and inter-telomere length as the denominator (for the entire chr. 1 and the short arm of chr. 2). Separate findings for both chromosomes 1 and 2p were included to provide an internal replication. Chromosome 1 was shown to be consistent with our earlier studies and chromosome 2p was used because the PNA probe for its chromosome 2 centromere provided easy identification. In addition, overall light intensity measures were obtained from interphase preparations, again based on 20 cells per participant prior to and following development of MCI-DS or dementia. Quantification of light intensity was also provided by the MetaSystems software (Fig. 1). Figure 1 is a FISH preparation of a metaphase derived from short-term lymphocyte cultures using phytohemagglutinin (PHA) from a person with DS and mild cognitive impairment. Figure 1 identifies chromosome 2 using a PNA (Peptide nucleic acid) probe specific for the centromere of chromosome 2, and measures its short arm in microns (micrometers) as explained in the Figure 1 legend. The short arm telomeres of chromosome 2 have also been measured by the PNA telomere probe and a MetaSystems ISIS image analyzer.

The interphase light intensity (ILI) was measured by the MetaSystem Image Analyzer and statistical comparisons were made using ILI units from averages of fluorescence intensities of PNA FISH probes labelled with FITC from individuals with DS but no MCI/dementia versus fluorescence intensities of samples from people with DS and MCI/dementia. People whose samples were analyzed for chromosome length in microns were analyzed using ratios of the overall chromosome 1 or chromosome 2p length in microns minus the telomere (probe) micron length divided into the total telomere length for chromosomes 1 and 2p.

TABLE I. Changes in Telomere Length as a Biomarker for Dementia Status

Case	Age (years)/Sex	Status	APOE	ILI[a] Interphase light intensity units	Chr 1[b] (chromosome 1)	Chr 2 p[c] Short arm of chromosome 2
(1)	49.8/f	No MCI-DS (Mild Cognitive. Impairment-MCI)	3/3	6.4	0.12	0.13
	54.6	MCI-DS		4.9 ($P < 0.005$)	0.08 ($P < 0.00001$)	0.09 ($P < 0.0002$)
(2)	52.9/f	No MCI-DS	3/3	19.0	0.16	0.22
	55.3	MCI-DS		6.2 ($P < 0.00001$)	0.08 ($P < 0.00001$)	0.11 ($P < 0.00001$)
(3)	58.8/m	No MCI-DS	3/3	11.6	0.17	0.21
	60.3	MCI-DS		4.6 ($P < 0.00001$)	0.1 ($P < 0.00001$)	0.12 ($P < 0.00001$)
(4)	48.4/f	No MCI-DS	3/4	11.9	0.17	0.21
	49.5	MCI-DS		4.4 ($P < 0.00001$)	0.08 ($P < 0.00001$)	0.11 ($P < 0.00001$)
(5)	46.0/m	No MCI-DS	3/3	13.3	0.15	0.18
	50.9	Dementia		4.3 ($P < 0.00001$)	0.1 ($P < 0.00001$)	0.1 ($P < 0.00001$)

[a]ILI from PNA probe(s).
[b]Chromosome 1 telomere length in microns/inter-telomere distance in μm.
[c]Chromosome 2p telomere length/Chromosome 2p length including the distal interface of the 2p cen PNA probe less the 2p telomere length.

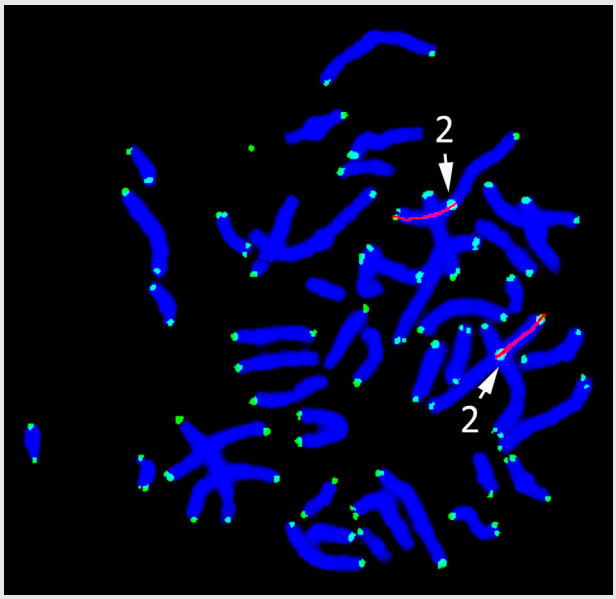

FIG. 1. Metaphase preparation from short-term T-lymphocyte cultures from a person with DS–MCI-DS, with a red line on both short arms of chromosome 2 (2p). The 2p at 3:00 is 5.9 μm and the 2p above it is 6.2 μm. Arrowheads point to both chromosomes 2. The telomeres and chromosome 2 centromeres are labeled by peptide nucleic acid probes and FITC with DAPI counterstaining. Linear measurements of the short arm of chromosome 2 were made using a *MetaSystems* image analyzer in combination with a *Zeiss Axioskop 2 plus*. Also the 2p telomere lengths were 0.9 μm at 3:00 in Fig. 1, while the upper and lower sister chromatid telomeres for the other chromosome 2 were 0.4 and 0.5 μm, respectively. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb]

RESULTS

Longitudinal Change in Telomere Length With Transition to Dementia

Table I above summarizes results for the five participants with DS prior to and following development of either MCI-DS or early dementia (with telomere data representing the means of 20 cell values). A first level of analysis employing *t*-tests for matched samples (df = 4) showed that telomere length was significantly shorter at Time 2 compared to Time 1 for all measurement methods, $4.1 < ts < 7.1$, $ps < 0.015$.

A second level of analysis focused on the significance of changes within individual participants, contrasting the 20 measurements at Time 1 with those at Time 2 employing *t*-tests for independent samples (df = 38). Again, all measurement methods showed that telomere lengths at Time 2 were consistently shorter than at Time 1, with probabilities indicated in Table I.

A final level of analysis entailed comparisons between the current study and "historical controls" available from our previous cross-sectional study of telomere length as a biomarker of MCI-DS. That previous study included 11 MCI-DS cases that should have telomere lengths comparable to the current Time 2 findings, along with 11 demographically matched controls that should show no differences between the current Time 1 findings. A set of six independent *t*-tests (df = 14) confirmed these null predictions (mean of the six $ts = 0.615$, $ps > 0.1$). Further, significant differences were found where expected ($3.6 < ts < 14.8$, $ps < 0.01$), the only exception being for the ILI measure at Time 1 compared to the previous 11 cases with MCI-DS, $t = 1.24$, *ns*.

DISCUSSION

Our previous studies [Jenkins et al., 2006, 2008, 2010, 2012b] were designed to determine if various methods for quantifying telomere length could differentiate between adults with DS with and without dementia, and subsequently with and without MCI-DS. Cumulatively, these studies included a total of 26 individuals with dementia or MCI-DS, together with a comparable number of their age- and sex-matched peers with a consensus classification of "Not demented/Aging normally". In summary, we found that some methods for quantifying telomere length produced non-overlapping distributions (e.g., direct physical measurement in μm) for adults with DS with and without MCI-DS or dementia. The present findings displaying telomere shortening accompanying change in clinical status reflective of AD progression complement those previous cross-sectional findings. Taken together, these results provide strong support for hypothesizing that measures of telomere length may serve as an informative biomarker for clarifying the dementia status of adults with DS, perhaps having near perfect, or even perfect sensitivity, and specificity. However, the association between telomere length and clinical status of adults with DS has been studied in a relatively small number of individuals to date, and additional studies are needed to confirm our results in a larger sample to allow sensitivity and specificity to be determined with greater precision and to allow systematic analyses of potential confounders (e.g., sex, ApoE genotype, Aβ blood levels, presence of other indications of atypical aging).

If our findings continue to hold, rapid translation into clinical practice would be expected to improve diagnostic accuracy for individuals with DS developing AD, this would contribute to improved planning to address support needs. For individuals with symptoms mimicking dementia, negative findings would encourage a search for the true cause of those symptoms and more effective treatment.

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