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Increased "absence" of telomeres may indicate Alzheimer's disease/dementia status in older individuals with Down syndrome

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ABSTRACT

We have reported previously that telomeres (ends of chromosomes consisting of highly conserved TTAGGG repeats) were shorter in metaphase and interphase preparations in T lymphocytes from short-term whole blood cultures of women with Down syndrome (DS) and dementia compared to age-matched women with DS but without dementia [E.C. Jenkins, M.T. Velinov, L. Ye, H. Gu, S. Li, E.C. Jenkins Jr., S.S. Brooks, D. Pang, D.A. Devenny, W.B. Zigman, N. Schupf, W.P. Silverman, Telomere shortening in T lymphocytes of older individuals with Down syndrome and dementia, Neurobiol. Aging 27 (2006) 41–45]. Our previous study was carried out by measuring changes in fluorescence intensity [using an FITC-labeled peptide nucleic acid (PNA) probe (Applied Biosystems; DAKO) and Applied Imaging software], and we now report on a substantially simpler metric, counts of signals at the ends of chromosomes. Nine adults with DS and dementia plus four who are exhibiting declines in cognition analogous to mild cognitive impairment in the general population (MCI-DS) were compared to their pair-matched peers with DS but without dementia or MCI-DS. Results indicated that the number of chromosome ends that failed to exhibit fluorescent signal from the PNA telomere probe was higher for people with dementia or mild cognitive impairment (MCI-DS). Thus, a simple count of chromosome ends for the "presence/absence" of fluorescence may provide a valid biomarker of dementia status. If this is the case, then after additional research for validation to assure high specificity and sensitivity, the test may be used to identify and ultimately guide treatment for people at increased risk for developing mild cognitive impairment and/or dementia.

Telomeres are chromosome ends that consist of highly conserved TTAGGG repeats and become shorter with every cell division. Increased telomere shortening has been associated with a variety of conditions including apoptosis and replicative cellular senescence [2,12], neoplastic transformation [20], in vivo cellular aging [13,17,1,9], heart disease [21,4], stress [8,6], osteoporosis [26], obesity [25], dyskeratosis congenita [27], and Alzheimer's disease (AD) [18]. The recent finding on UUAGGG-repeat telomeric RNAs may provide better understanding of the role of the telomere in the above conditions [22].

We have previously observed quantitatively reduced telomere size in metaphase and interphase preparations from short-term whole blood cultures of adults with DS and dementia compared to age-matched adults with DS and no dementia [14,15]. In addition we have broadened our original study to show that telomere length is also shortened for people with DS who exhibited cognitive declines analogous to mild cognitive impairment (MCI) in the general population, referred to as MCI-DS in the present study. MCI is defined as an intermediate stage between cognitive declines typical of brain aging, *per se*, and the deficits that occur with dementia, during which daily living activities are generally unaffected (e.g. [11]). Declines observed are not sufficiently severe to meet criteria diagnostic for dementia [19]. Individuals with MCI are more likely to convert to dementia than peers without MCI [23], especially those with memory function decline. Similar to

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dementia diagnosis, MCI identification in adults with DS is complicated by lifelong cognitive deficits and substantial inter-individual variability in baseline abilities. However, longitudinal assessments of cognition can be used to characterize changes suggestive of MCI within this population, and that was the procedure employed in this study (see [7,16,29]).

Quantitative measurement of telomere length requires the use of sophisticated and specialized methods and equipment. Therefore, we wanted to determine if a simpler metric could distinguish adults with dementia or MCI-DS from their unaffected peers with DS. For this purpose, we chose counts of signals (present/absent) from fluorescently tagged chromosome ends (employing an FITC-labeled peptide nucleic acid probe).

Adult subjects with DS were recruited using an Institutional Review Board-approved protocol with correspondent-informed consent and participant assent. A community-based sample of 234 women with DS, 45–78 years of age, and 124 men from 45 to 73 years of age, was obtained through the New York State Developmental Disability Service System or direct contacts with provider agencies in neighboring states. These people have been participating in a longitudinal study examining changes in functioning associated with aging and dementia in adults with intellectual disability and have been evaluated at 14–18 month intervals to determine cognitive, functional and health status (see e.g. [28]). From this larger sample, 11 pairs of females, as well as two pairs of males (all with complete trisomy 21) were age-matched, one individual within each pair having dementia or MCI-DS and the other not. Samples from females have been more available than males because of an ongoing project focused on women's health [27]. Table 1 indicates the age, sex, and dementia status of individuals within our sample.

The dementia status of each participant was determined at a consensus conference which included all senior staff members participating in our longitudinal assessments and research assistants who had direct contact with the participants under consideration [24,29]. Participants were classified based upon consideration of information available from a detailed review of clinical records, informant interviews, and direct assessments of selected cognitive functions, including evidence of decline over the 14- to 20-month period between assessments. Dementia status was classified consistent with diagnostic guidelines recommended by the AAMR-IASSID Working Group for the Establishment of the Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability [3,5], that were based upon current ICD-10 criteria (WHO, 1992). Each case was classified as: (a) *non-demented,* indicating with reasonable certainty that significant age-associated impairment was absent; (b) MCI-*DS status,* indicating that there was substantial uncertainty regarding dementia status, with some indication of mild cognitive and/or functional decline but importantly, the observed change(s) did not meet dementia criteria; (c) *possible dementia,* indicating that some signs and symptoms of dementia were present, but declines over time were not judged to be totally convincing, although there was more certainty than for the MCI-DS status classification; (d) *definite dementia,* indicating with reasonable confidence that dementia was present based upon substantial decline over time and absence of other conditions that might mimic dementia (e.g., untreated hypothyroidism). For each participant who was rated as having possible or definite dementia, findings were reviewed to establish a differential diagnosis. These were either AD or AD in combination with possible other cause(s) (e.g., Parkinson's disease) given the substantial AD neuropathology characteristic of DS at these ages. Participants could also be categorized as (e) *status uncertain due to complications,* indicating that the criteria for possible dementia had been met, but symptoms might be caused by some other substantial concern, usually a medical condition unrelated to a dementing disorder (e.g., severe sensory loss, poorly resolved hip fracture and psychiatric diagnosis), and (f) *indeterminable,* indicating that the pre-existing disability was of such severity that detection of decline indicative of dementia was not possible (e.g., profound ID with multiple handicaps). Participants in these latter two categories were not included in the present study.

Table 1
The mean number of chromosome arms with no signal (MNCANS) for studies 1–13 showing greater loss of signal associated with dementia and MCI-DS among 26 people with DS

Study	Sex	Age	Dementia status[a]	MNCANS	*p*
1	F	58.3	PD	19.0 (7.3)[b]	
1	F	60.3	ND	11.0 (7.2)	<.002
2	F	53.8	DD	13.1 (5.5)	
2	F	57	ND	7.7 (5.6)	<.004
3	F	58.8	PD	13.1 (6.0)	
3	F	60.6	ND	7.3 (4.1)	<.001
4	M	62.5	DD	9.4 (4.8)	
4	M	63.2	ND	5.2 (3.9)	<.004
5	M	58	PD	13.0 (5.7)	
5	M	64.5	ND	4.2 (3.0)	<.000001
6	F	59	DD	16.4 (7.1)	
6	F	65.4	ND	5.6 (3.7)	<.000001
7	F	66.9	PD	18.3 (6.1)	
7	F	69.8	ND	6.6 (3.7)	<.000000
8	F	57	DD	10.3 (4.4)	
8	F	58	ND	4.3 (2.3)	<.000004
9	F	55.8	DD	16.2 (9.7)	
9	F	57.8	ND	3.9 (4.1)	<.000007
10	F	49.8	MCI	8.6 (5.2)	
10	F	50.2	ND	8.0 (4.2)	<.7
11	F	54.2	MCI	9.1 (6.0)	
11	F	54.6	ND	6.1 (5.1)	<.1
12	F	51.5	MCI	12.0 (3.5)	
12	F	53	ND	5.9 (2.7)	<.000000
13	F	53	MCI	10.0 (3.7)	
13	F	53.3	ND	4.2 (2.8)	<.000004

[a] DD, PD, ND, and MCI = definite dementia, probable dementia, no dementia, and mild cognitive impairment, respectively.

[b] 19.0 (7.3) = mean number of signals lost with a standard deviation of 7.3.

Telomeres in metaphase spreads (T lymphocytes from freshly collected whole blood) were hybridized using an FITC-labeled peptide nucleic acid probe and DAPI counterstaining [14]. The actual number of fluorescent signals for each metaphase was determined by simply counting the signal number per metaphase and tabulating the number of chromosome arms that did not exhibit an FITC signal for each participant.

Fig. 1 shows an image of a metaphase from a short-term whole blood culture, hybridized with an FITC-labeled PNA probe such that telomeres are labeled at most metaphase chromosome ends and within the interphase nucleus. The results of within-pair comparisons of the number of chromosome arms with no fluorescent signal are given in Table 1.

Results, summarized in Table 1, were generated by counting the number of chromosome arms with no "visible" telomeres in each of 20 metaphase cells per subject. Overall, the distributions of scores for individuals with dementia or MCI-DS versus their non-demented peers had almost no overlap, $t(12) = 7.18$, $p < .00003$. In fact, all individuals with dementia or MCI-DS had means of over eight missing signals while that was true for only a single non-demented individual. The difference between affected and unaffected individuals was also significant for pairings with just demented cases or just MCI-DS cases, $t(8) = 8.19$, $p < .0005$ and $t(3) = 2.99$, $p < .03$ (one-tailed), respectively, but a mixed model analysis of variance (with diagnosis as a between subjects variable and affected/unaffected as a within subjects variable) indicated that

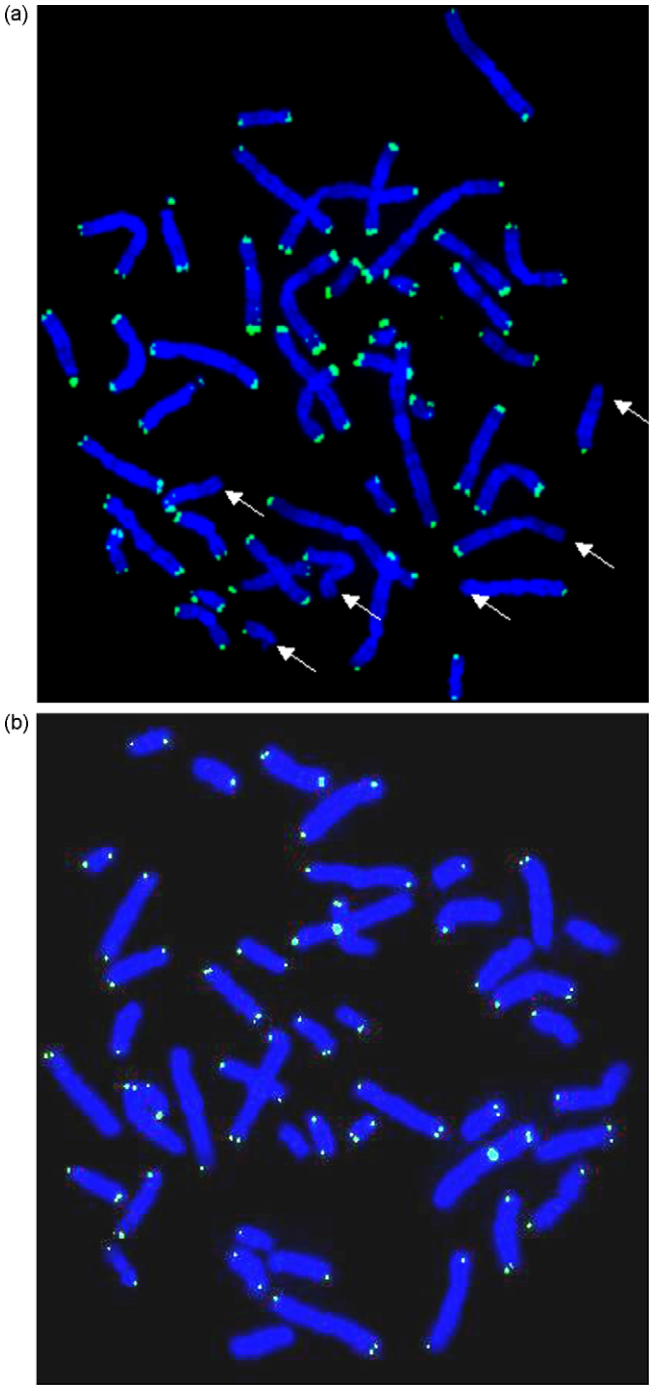

Fig. 1. (a) Telomeres in metaphase shown by an FITC-labeled PNA probe with DAPI counterstaining. Examples of chromosome arms with no signal are shown by arrows. This cell was obtained from a whole blood culture of a person with Down syndrome with no dementia/MCI. (b) Similar to Fig. 1a except there are more signals (over 20) missing from this metaphase obtained from a whole blood culture of a person with MCI-DS.

signal loss was greater in cases with dementia compared to MCI-DS, $F(1,11) = 6.02$, $p < .032$ (see Fig. 2).

While presence/absence of signal represents a relatively simple method of measurement, it is important to note that this measure is an imperfect reflection of true telomere status. Factors unrelated to telomere length could reduce a signal below the detection threshold of our equipment, and therefore some telomeres may actually

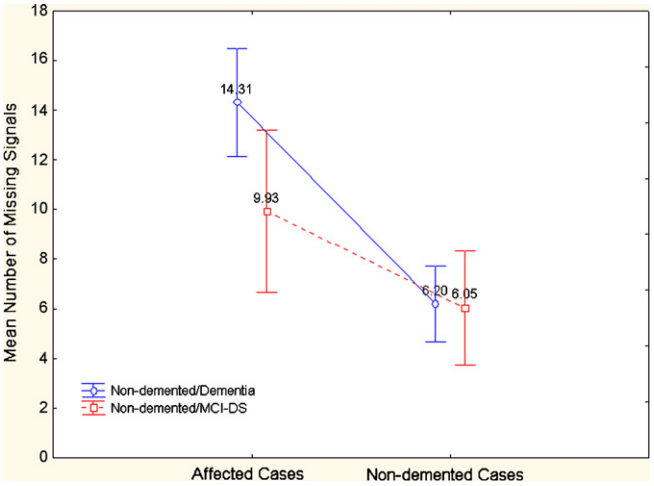

Fig. 2. Telomere fluorescent signal loss: interaction showing greater effect with dementia compared to MCI-DS. Interaction effect: $F(1,11) = 6.02$, $p = .032$.

be present and functioning where no signal was observed. Nevertheless, it seems safe to assume that any extraneous factors should have equal influences on individuals with or without dementia or MCI-DS, and any differences in signal number associated with dementia status should be a valid indication of differential telomere length.

These results not only show that the number of "visible" telomere signals is significantly less in people with DS and dementia versus those in age- and sex-matched controls with DS only, and they suggest that adults with DS without dementia can be distinguished from adults with DS experiencing cognitive decline presumably associated with the progression of AD simply by counting the number of chromosome arms with no signal from the FITC-labeled PNA telomere probe. If these results are confirmed in a larger sample, findings will provide the foundation for a diagnostic procedure having both high sensitivity and specificity. As already mentioned, shorter telomeres have been found in people with AD compared to controls [18,10] and in people with DS and dementia and/or MCI-DS versus people with DS only [14], and recently shorter telomeres have been observed in people with reduced immune function who are caregivers of patients with AD [6]. It will be interesting and exciting to determine whether the loss of signals that we have observed can be directly correlated with increased telomere shortening in these conditions. Only additional research will answer this question.

Since there is as yet no clear biomarker for dementia/Alzheimer's disease, a longitudinal study would be useful to determine whether reduced signal number in individuals with DS precedes clinical signs of dementia, and to determine the underlying mechanism responsible for this association. Recognition of pre-clinical and early dementia would be very useful in this population of adults who already have pre-existing cognitive impairments. Early detection is especially important because it would allow earlier treatment and justify the initiation of intervention strategies soon enough to minimize damage to the central nervous system. Further, a valid biomarker would be of great value for differential diagnosis, given the variety of conditions that can cause behavioral or cognitive changes in older adults with DS.

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