Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2012 July ; 159B(5): 598–604. doi:10.1002/ajmg.b.32066.

Mild cognitive impairment identified in older individuals with Down syndrome by reduced telomere signal numbers and shorter telomeres measured in microns

Edmund C. Jenkins^{1,*}, Lingling Ye¹, Milen Velinov¹, Sharon J. Krinsky-McHale¹, Warren B. Zigman¹, Nicole Schupf^{1,2}, and Wayne P. Silverman³

¹New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, United States

²Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, 630 West 168th St., New York, NY 10032, United States

³The Kennedy Krieger Institute and The Johns Hopkins University School of Medicine, Suite 222s, 707 North Broadway, Baltimore, MD 21205, United States

Abstract

Previously, we established that short-term T lymphocyte cultures from people with Down syndrome (DS) and dementia (Alzheimer's Disease) had shorter telomeres than did those from age- and sex- matched people with Down syndrome only, quantified as significantly reduced numbers of signals of PNA telomere probes in whole metaphases [Jenkins et al., 2008] as well as reduced telomere probe light intensity values in interphases [Jenkins et al., 2010]. We now describe shorter telomere length in adults with DS and mild cognitive impairment (MCI) compared to age- and sex-matched individuals with DS without MCI. Telomere length is quantified by reduced telomere signal numbers and shorter chromosome 1 telomeres measured in micrometers (microns). These findings were in agreement with quantitative light intensity measurements of chromosome 1 and chromosome 21 PNA telomere probes with and without the use of a "normalizing ratio" involving the fluorescence exhibited by a PNA probe for centromere 2, and with the use of light intensity measurements of interphase preparations. Most importantly, the distributions of chromosome 1 telomere lengths (in microns) were completely non-overlapping for adults with and without MCI, indicating that this measure has great promise as a biomarker for MCI as well as dementia in this population.

Keywords

Down Syndrome; Mild Cognitive Impairment; FISH; PNA telomere signal number; Telomere Length in Microns

INTRODUCTION

Down syndrome (DS) is the most prevalent chromosomal cause of intellectual disability, currently affecting one in every 691 newborns [Parker et al., 2010]. As adults, people with

*Correspondence to: Edmund C. Jenkins, Ph.D., Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314. edjenkins.jenkins85@gmail.com; Ed.Jenkins@opwdd.ny.gov.

Disclosure statement

There are no actual or potential conflicts of interest among the authors of this article.

DS are at greatly increased risk for dementia/Alzheimer's disease (AD) [Coppus et al., 2006; Zigman and Lott, 2007]. We have established that individuals with DS and AD (AD-DS) have shorter telomeres (chromosome ends consisting of highly conserved TTAGGG repeats) than their sex- and age-matched peers with DS without AD [Jenkins et al., 2006, 2010]. We have also shown that these effects could be reliably obtained both by measuring light intensity quantitatively or by simply counting the number of signals (fluorescent signals from the PNA probe specific for the telomere region) and calculating "missing" telomeres [Jenkins et al., 2008].

Because early diagnosis will become even more important as effective treatments for AD are discovered, we conducted a preliminary study to determine if telomere shortening might also occur in adults with DS and mild cognitive impairment (MCI), a condition associated with declines that are of insufficient severity to warrant a diagnosis of dementia [Winblad et al., 2004]. Those results suggested that MCI might indeed be associated with telomere shortening [Jenkins et al., 2010]. Given the challenges associated with recognizing declines in cognition against the background of lifelong impairments that are part of the DS phenotype, a valid biomarker would be particularly useful for reducing diagnostic uncertainty for this high-risk patient population. Therefore, the present study was conducted with a larger sample and a broader range of measurement methods.

MATERIALS AND METHODS

Human Subjects

Whole blood samples were obtained from a subsample of 22 individuals chosen from among a cohort of 406 men and women with DS ranging in age from 42 to 80. The larger sample included all participants in a larger longitudinal study of aging and dementia within this target population [Silverman et al., 2004]. All participants were recruited through organizations serving people with intellectual disability and operating in New York or the broader New York City metropolitan area. All study enrollment and assessment procedures were approved by IRBs at the investigators' respective institutions, and required consents and assents were obtained for every participant.

Assessments were conducted at 14–20 month intervals over 5 cycles of data collection and included examination of clinical and psychosocial records, interviews with knowledgeable informants, and direct testing of cognitive abilities. Following completion of each cycle of data collection, the dementia status of each participant was determined at a consensus conference which included all senior staff members participating in our longitudinal studies and research assistants who had direct contact with the participants under consideration [Silverman et al., 2004; Zigman et al., 2004]. Participants were classified based upon consideration of all information available from the detailed review of clinical records, informant interviews, and direct assessments of cognition, including evidence of decline between assessments, consistent with the recommendations of the AAMR-IASSID Working Group for the Establishment of the Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability [Aylward et al., 1997; Burt and Aylward, 2000]. Each case was classified as: (a) *non-demented*, indicating with reasonable certainty that significant age-associated impairment was absent; (b) *MCIDS*, indicating that there was some indication of mild cognitive and/or functional decline but 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 sufficiently convincing to classify "definite" dementia; (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, psychiatric diagnosis), and (f) *indeterminable*, indicating that the preexisting disability was of such severity that detection of decline indicative of dementia was not possible [e.g., profound ID (Intellectual Disabilities) with multiple handicaps]. For the present study, only participants classified as non-demented or MCIDS were eligible for inclusion.

Blood Samples

"Buffy coats" (defined in this context as the mononuclear cells obtained via Ficoll Paque gradient centrifugation) were generated and cryopreserved until we had sufficient samples from people with MCIDS who were age- and sex-matched to non-demented cases. The blood samples were drawn and MCI status (present/absent) was determined during the same assessment cycle within our larger longitudinal study (see Silverman et al. 2004). Among the 11 pairs of cases, 7 were women and 4 were men, with mean ages of 55.86 and 54.54 years, respectively. Whole blood samples sent to our lab for telomere studies were rendered anonymous by staff from our larger longitudinal program not otherwise involved in the present study. In addition, evaluations of telomere length were blinded with respect to clinical status.

Heparinized whole blood was processed immediately or held overnight at room temperature until buffy coats could be generated and frozen in liquid nitrogen (10% DMSO in fetal bovine serum). Telomeres were evaluated following rapid thawing and short-term culturing of the samples for 4 days at 37°C. Initial cultures contained 200,000–400,000 cells per ml, previously obtained via Ficoll Paque gradient centrifugation and frozen in liquid nitrogen until age- and sex-matched pairs of samples became available from individuals with no signs of MCI/AD-DS, as well as those who had converted to MCIDS, all studied clinically over periods of 14–18 months.

Telomere Studies – light intensity, linear and signal number analyses

Changes in fluorescent light intensities as well as direct linear measurements in microns were used to detect changes in telomere size [Jenkins et al., 2006, 2010] using a MetaSystems image analyzer (Waltham, MA). A FITC-labeled PNA probe (DAKO, North America) was used as previously described [Lansdorp et al., 1996; Londoño-Vallejo, 2001; Jenkins et al., 2006, 2008, 2010] as well as a cen 2 PNA probe (a gift for investigational use from DAKO, Glostrup, Denmark). The cen 2 data provided a non-telomere standard that allowed us to generate light intensity ratios from telomere intensities/cen 2 intensities [Perner et al., 2003]. While we have obtained virtually the same results with and without the use of a cen 2 control, we recognize that light intensities can vary from preparation to preparation or from lot- to-lot of PNA probe, for example, so that using a probe for cen 2, which is not expected to be affected by age or dementia status, provided additional experimental control. Similar to cen 2 ratio analysis, we used another ratio for "direct" linear analysis in microns by dividing the total telomere length for chromosome 1 by the total chromosome length for chromosome 1 minus the total telomere length.

All light intensity data were generated by the image analyzer, while measurements of absolute physical length (in microns) and numbers of chromosome arms with no signals entailed locating metaphases by a microscopist. Manual counts were made of missing telomeres while measures of physical length (in pixels) were determined using MetaSystems

Image Analyzer software (called “Isis”). Those pixel counts were then converted to microns for statistical analysis. Interphase preparations were also examined and shown to be effective for identifying people with MCI, given that sample size may sometimes be insufficient to obtain an adequate number of metaphase preparations for light intensity and/or signal number analysis (cumulative experience given in Jenkins et al., 2006, 2008, 2010, and Table 1 below).

Metaphase chromosomes 1, 21, interphase preparations, PNA telomere probe signal numbers, and chromosome 1 telomere linear length

The above-mentioned chromosomes were examined in this study based on our previous findings [Jenkins et al., 2010] and because using a single chromosome reduced overall turn-around time per study. For each individual, twenty metaphase chromosome preparations from short-term T-lymphocyte cultures from cryogenically archived buffy coats were analyzed blind to dementia status, separately for chromosomes 1 and 21 followed up by cen 2 ratio analyses. After two specimens (one classified as MCIDS and the other as non-demented) were matched by age and sex, the samples were coded to ensure that telomere measurements were conducted blind to clinical status. Two slides were made from each sample that were then hybridized with the PNA probes and counterstained. Twenty metaphases were chosen at random, digitized and uploaded into the MetaSystems image analyzer and light intensities were then measured by the system’s computer software.

In addition, light intensity measures were obtained from interphase preparations and the number of chromosome arms with missing PNA telomere signals was also determined. Finally, direct measurements (in microns) of chromosome 1 telomeres were obtained and used to examine differences in telomere length. All light intensity and linear measurements were done with MetaSystems software, while a simple fluorescence microscope was all that was needed to detect PNA probe signal differences [Jenkins et al., 2008].

RESULTS

Individual chromosomes 1 and 21 and cen 2 ratio analysis

Shortened telomeres were found in individual chromosomes 1 and 21 in a total of all 11 individuals with DS/MCI compared to matched controls, both with and without adjusting for cen 2 light intensity. Table I summarizes findings for chromosomes 1 and 21, with and without cen 2 ratios (t test P values $<.004$). Results were essentially unchanged for ratios with cen 2 intensity, except that using this measure generally resulted in increased statistical significance. Cumulatively then, we have established that telomeres were shorter in individuals with MCI and DS versus those with DS alone.

Interphase telomere light intensity measurements, PNA telomere probe signal analyses, and linear measurements in microns

Using the same material from the 11 individuals with MCI, we have also identified individuals with DS/MCI by detecting: (1) shorter telomeres in interphase preparations from short-term T lymphocyte cultures (p values $<.04$), (2) reduced PNA telomere signal numbers in all pairings for DS/MCI ($P<10^{-6}$ for 10 of 11 cases; see MNCANS data in Table I that shows the mean number of chromosome arms with no signal was 10.6 for controls versus 24.1 for MCIDS samples), and finally (3) reduced telomere length in T lymphocyte cultures from people with DS/MCI by measuring absolute chromosome 1 telomere length (as indicated by the telomere PNA probe) in terms of microns (See Figs. 1 and 2, and Table I.) ($P<10^{-6}$ for all 11 pairings).

While comparisons between cases with MCI and their individually matched pairs confirm that measures of telomere length are sensitive to risk of developing AD in the population with DS, they have limited practical utility as biomarkers because contemporaneous samples from a matched control would rarely be available, if ever. Therefore, we conducted additional analyses to examine the degree to which distributions of scores for the group with MCIDS overlapped with that of non-demented adults with DS. The bottom of Table 1 provides a summary of these results. As expected, all measures showed shorter mean telomere lengths for the group with MCI, $3.30 < t_s(20) < 15.71$, $P_s < .005$; Mann-Whitney U $s < 15.5$, $P_s < .004$. However, the various measures differed in the degree to which the groups overlapped. All direct measures based on light intensity showed small overlaps in group distributions, indicating that any single criterion score would have imperfect sensitivity or specificity. The distributions of ratios of light intensity measures standardized to the Chromosome 2 centromere showed complete group separation, with the difference between the largest values for a case with MCIDS (0.8 – 0.9) and the smallest values for a non-demented case (1.0 – 1.1) in the range of 1 S.D. (0.2).

Measures of “direct” telomere (PNA probe) length also showed complete group separation, with a more substantial gap between distributions. In fact, the longest MCIDS (3.1 microns) and shortest control (3.9 microns) scores were well over a standard deviation (0.4 – 0.6 microns) apart. In the case of the ratio measure using the remainder of chromosome 1 as a standard, the separation between the distribution extremes (0.14 versus 0.20) increased to approximately 4 SDs. That remarkable finding suggests that a ratio classification criterion of 0.12 would have perfect sensitivity and specificity, even taking measurement imprecision into account. (Table I also provides a “best” classification criterion for each measure, along with sensitivity and specificity estimates.)

4. CONCLUSIONS/DISCUSSION

We have detected consistently shorter telomeres associated with MCI in older adults with DS using 8 methods/measurements: (1) chromosome 1 telomere light intensity alone; (2) chromosome 21 telomere light intensity alone; (3) chromosome 1 telomere: cen 2 light intensity ratio; (4) chromosome 21 telomere: cen 2 light intensity ratio; (5) total telomere light intensities measured in interphase; (6) numbers of chromosome arms with no detectable signal; (7) actual physical lengths of chromosome 1 telomeres; and (8) ratios of chromosome 1 telomere length to the remainder of chromosome 1. As expected based on previously reported preliminary results [Jenkins et al, 2010], all measures included in the present study indicated that MCI was associated with telomere shortening. However, there were important differences among these methods with respect to the differentiation between individuals with and without MCIDS, suggesting that some specific measures have greater promise than others as biomarkers of clinical status.

For all “simple” measures of light intensity (1, 2, 5, and 6, above), some minimal overlap in measurements was observed between groups, indicating that any specific measurement value chosen as a classification criterion would have excellent but imperfect specificity or sensitivity. The use of the cen 2 probe as a standard eliminated these overlaps and represented a step in the right direction, but the separation between groups of approximately 1 S.D. still suggests that a best case criterion, while having excellent validity, would again be unlikely to have perfect sensitivity and specificity.

Our findings for “direct” linear measurements in microns were more promising. Just over 2S.D.s separated the longest chr 1 telomere length from a case with MCIDS (3.1 microns) from the shortest non-demented case (3.9 microns), and this separation increased to over 4S.D.s when the remainder of chr 1 was used as a standard (MCIDS = 0.14 and Non-

demented = 0.20, S.D. = 0.012; see Table 1). This large degree of separation between distributions for affected and unaffected individuals suggests that a classification criterion of 0.17 for this ratio measure is very likely to have near perfect sensitivity and specificity throughout the population of older adults with DS. Of course, that value will need to be confirmed in a validation study, but even should adjustment be needed, it seems clear that this method of measurement will provide an informative biomarker of clinical status for this high-risk and diagnostically challenging population. This would be especially important for differential diagnosis in situations where a treatable condition presents clinically as a pseudo-dementia, but even in true cases of Alzheimer's disease, the objective confirmation of status would allow more effective planning for support needs.

In addition, our experience indicates that the physical measurement of chromosome length also had a relatively fast turnaround time, with data acquisition completed in approximately 6 hours for an individual case. Only light intensity telomere analysis of interphase preparations required less time, but these preparations had reduced sensitivity (Table I). It is possible (future studies are needed to validate this opinion) that only short-arm linear measurements could be used (for chromosome 2, for example, already identified with the cen 2 probe) and thus significantly reduce turnaround times. Physically measuring the telomere length in microns should also be feasible without the expensive software required to quantify light intensity. Most simply, the magnified images of chromosomes could be printed and telomere lengths could be measured with a precision caliper. Alternatively, the PNA telomere probe might be labeled with horse radish peroxidase, visualized using bright field microscopy, and measurements could be made from either black and white images on prints or directly, with the use of a stage and ocular micrometer.

These findings provide compelling evidence that shorter telomere length in short-term T lymphocyte cultures from people with DS can serve as a biomarker for the presence of MCI as well as dementia [Jenkins et al, 2006]. While the empirical association between telomere length and clinical status is clearly evident within the older populations of adults with DS (Jenkins et al., 2008, 2010, present study), the biological mechanism(s) responsible for this association remain unknown. In fact, we do not yet know if shorter telomeres represent a risk factor, with individuals having shorter telomeres more likely to develop dementia, or a biomarker, with telomere length decreasing as the underlying Alzheimer's disease progresses. Additional longitudinal assessments of telomere length are needed to distinguish between these two possibilities.

The association between telomere length and clinical status does not appear to be unique to DS. Panossian et al. (2003) first described a comparable relationship for the general population. Subsequent studies confirmed these findings with respect to dementia although results focusing on MCI have been mixed (Hochstrasser et al., 2012; Movérare-Skrtic et al., 2012). Our study has been limited to people with DS and may not be generalizable to other populations, but we believe it adds to the evidence indicating that measures of telomere length in T cells can be an informative part of diagnostic evaluations in all cases of suspected AD.

Acknowledgments

Thanks are due the research participants and various cooperating agencies as well as all staff involved in this project. ECJ also personally thanks André T. Hoogeveen, Ph.D., Department of Clinical Genetics, Erasmus University in Rotterdam, for suggesting the "physical" measurement of telomeres. We also acknowledge the help of Mr. Ezzat El-Akkad of the Graphic Arts Department and Mr. Lawrence Black, Institute Librarian. This work was supported in part by the NYS OPWDD Institute for Basic Research in Developmental Disabilities, Alzheimer's Association grants IIRG-07-60558, IIRG-96-077; and NIH grants PO1-HD35897, RO1-HD37425, and RO1-AG014673.

Grant sponsor: New York State Office for People with Developmental Disabilities; Grant sponsor: Alzheimer's Association; Grant numbers: IIRG-07-60558, IIRG-96-077; Grant sponsor: NIH; Grant numbers: PO1-HD35897, RO1-HD37425, RO1-AG014673, and P30-HD024061.

References

- Aylward E, Burt D, Thorpe L, Lai F, Dalton A. Diagnosis of dementia in individuals with intellectual disability. *J Intellect Disabil Res.* 1997; 41:621–664.
- Burt DB, Aylward E. Test battery for the diagnosis of dementia in individuals with intellectual disability. *J Intellect Disabil Res.* 2000; 44:262–270.
- Coppus A, Evenhuis H, Verberne GJ, Visser F, van Gool P, Eikelenboom P, van Duijn C. Dementia and mortality in persons with Down's syndrome. *J Intellect Disabil Res.* 2006; 50:768–777. [PubMed: 16961706]
- Hochstrasser T, Marksteiner J, Humpel C. Telomere length is age-dependent and reduced in monocytes of Alzheimer patients. *Exp Gerontol.* 2012; 47(2):160–163. [PubMed: 22178633]
- Jenkins EC, Velinov MT, Ye L, Gu H, Li S, Jenkins EC Jr, Brooks SS, Pang D, Devenny DA, Zigman WB, Schupf N, Silverman WP. Telomere shortening in T lymphocytes of older individuals with Down syndrome and dementia. *Neurobiol Aging.* 2006; 27:941–945. [PubMed: 16046031]
- Jenkins EC, Ye L, Gu H, Ni SA, Duncan CJ, Velinov MT, Pang D, Krinsky-McHale SJ, Zigman WB, Schupf N, Silverman WP. Increased "absence" of telomeres may indicate Alzheimer's disease/ Dementia status in older individuals with Down syndrome. *Neurosci Lett.* 2008; 440:340–343. [PubMed: 18571319]
- Jenkins EC, Ye L, Gu H, Ni SA, Velinov M, Pang D, Krinsky-McHale SJ, Zigman WB, Schupf N, Silverman WP. Shorter telomeres may indicate dementia status in older individuals with Down syndrome. *Neurobiol Aging.* 2010; 31:765–771. [PubMed: 18635289]
- Lansdorp PM, Verwoerd NP, van de Rijke FM, Dragowska V, Little M-T, Dirks RW, Raap AK, Tanke HJ. Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet.* 1996; 5:685–691. [PubMed: 8733138]
- Londoño-Vallejo JA, DerSarkissian H, Cazes L, Thomas G. Differences in telomere length between homologous chromosomes in humans. *Nucleic Acids Res.* 2001; 29:3164–3171. [PubMed: 11470873]
- Movérare-Skrtic S, Johansson P, Mattson N, Hansson O, Wallin A, Johansson JO, Zetterberg H, Blennow K, Svensson J. Leukocyte telomere length (LTL) is reduced in stable mild cognitive impairment but low LTL is not associated with conversion to Alzheimer's disease: a pilot study. *Exp Gerontol.* 2012; 47(2):L:179–L:182.
- Panosian LA, Porter VR, Valenzuela HG, Znju X, Reback E, Masterman D, Cummings JL, Effros RB. Telomere shortening in T cells correlates with Alzheimer's disease status. *Neurobiol. Aging.* 2003; 24:77–84. [PubMed: 12493553]
- Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A. National Birth Defects Prevention Network. Updated national birth prevalence estimates for selected birth defects in the United States: 2004–2006. *Birth Defects Res A Clin Mol Teratol.* 2010; 88:1008–1016. [PubMed: 20878909]
- Perner S, Brüderlein S, Hasel C, Walbel I, Holdenried A, Giloglu N, Chopurian H, Nielsen KV, Plesch A, Högel J, Möller P. Quantifying telomere lengths of human individual chromosome arms by centromere-calibrated fluorescence in situ hybridization and digital imaging. *Am J Pathol.* 2003; 163:1751–1756. [PubMed: 14578175]
- Silverman W. Incidence and prevalence of dementia in elderly adults with mental retardation without Down syndrome. *Am J Ment Retard.* 2004; 109:126–141. [PubMed: 15000676]
- Silverman W, Schupf N, Zigman W, Devenny D, Mizejeski C, Schubert R, Ryan R. Dementia in adults with mental retardation: assessment at a single point in time. *Am J Ment Retard.* 2004; 109:111–125. [PubMed: 15000666]
- Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LA, Nordberg A, Backman L, Albrecht A, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack K, van Duijn C, Visser P, Petersen RC. Mild cognitive impairment – beyond

controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *J Intern Me.* 2004; 256:240–246.

Zigman WB, Lott IT. Alzheimer's disease in Down syndrome: Neurobiology and risk. *Ment Retard Dev Disabil Res Rev.* 2007; 13:237–246. [PubMed: 17910085]

Zigman WB, Schupf N, Devenny DA, Mizejeski C, Ryan R, Urv TK, Schubert R.

Fig. 1. Metaphase preparation from short-term T lymphocyte cultures from a person with DS-MCI, with telomeres and cen 2 labeled (see arrowheads) by a peptide nucleic acid probe and FITC with DAPI counterstaining, for both metaphase and interphase. Linear measurements of the whole chromosome 1 and its telomeres made using a MetaSystems image analyzer. The length of the chromosome 1 at 8:00 was 14.9 μ , while the length of the other one was 14.0 μ .

Fig. 2.

Same cell as Fig. 1 except chromosome 1 telomeres have been measured as indicated. The upper (1p) and lower (1q) telomeres for the no. 1 chromosome at 8:00 were 0.5 and 0.7 μ , respectively, while the lengths of the upper (1q) and lower (1p) telomeres for the other chromosome 1 were 0.5 and 0.6 μ , respectively.

Table 1

Shorter telomeres from individuals with Down syndrome(DS) and MCI versus those with DS only, detected by telomere PNA probe light intensity differences on chromosome 1, chromosome 21, interphase preparations, reduced PNA probe signal numbers (MNCANS), and linear differences in micrometers (on Chromosome 1

Case No. ¹	Age (yrs)	Chr.1 ²	P ³ ×10 ⁻⁶	Cent 2 Ratio ⁴	P× 10 ⁻⁶	Chrom 21	P ×10 ⁻⁶	Cent2 Ratio	P ×10 ⁻⁶	Inter-phase	P ×10 ⁻⁶	MNCANS ⁵	P ×10 ⁻⁶	Chr. 1 tel.length in μm	P× 10 ⁻⁶	Ratio ⁶	P ×10 ⁻⁶
1(C) ⁷	55.8	5.8(2.7)	-	1.0(0.4)	-	6.2(2.6)	-	1.1(0.5)	-	15.1(6.5)	-	8.3(4.5)	-	3.9(0.6)	-	0.20(0.04)	-
1 (M) ⁸	53.3	2.5(1.0)	140	0.4(0.2)	280	1.7(1.0)	4	0.3(0.2)	3	8.4(5.4)	11×10 ²	33.4(9.5)	∞	1.5(0.4)	∞	0.11(0.04)	∞
2C	58.7	8.4(2.4)	-	1.6(0.2)	-	8.8(2.7)	-	1.6(0.3)	-	22.7(13.5)	-	8.2(3.1)	-	4.2(0.7)	-	0.20(0.04)	-
2M	56.4	3.1(1.9)	∞	0.7(0.3)	∞	3.3(1.3)	∞	0.7(0.2)	∞	15.1(6.6)	3×10 ⁴	33.3(7.5)	∞	1.8(1.0)	∞	0.11(0.03)	∞
3C	53.3	7.3(2.4)	-	1.4(0.3)	-	7.8(2.3)	-	1.4(0.3)	-	17.7(5.8)	-	7.4(2.5)	-	4.9(0.7)	-	0.22(0.04)	-
3M	52.3	2.8(0.9)	∞	0.6(0.1)	∞	3.1(1.0)	∞	0.7(0.1)	∞	6.0(2.6)	∞	21.1(4.5)	∞	2.4(0.6)	∞	0.11(0.03)	∞
4C	56	6.4(1.6)	-	1.1(0.1)	-	6.9(1.7)	-	1.2(0.1)	-	20.5(6.8)	-	7.1(2.0)	-	4.1(0.7)	-	0.20(0.04)	-
4M	55.3	3.2(0.8)	∞	0.8(0.1)	∞	3.4(0.9)	∞	0.8(0.1)	∞	6.2(2.8)	∞	16.5(3.5)	∞	2.7(0.6)	∞	0.11(0.03)	∞
5C	54.3	4.9(1.2)	-	1.4(0.3)	-	5.5(1.0)	-	1.6(0.4)	-	11.2(3.4)	-	8.9(1.9)	-	4.3(0.7)	-	0.21(0.04)	-
5M	54.6	2.9(0.7)	∞	0.7(0.1)	∞	3.2(0.9)	∞	0.8(0.1)	∞	4.9(1.2)	∞	20.4(4.3)	∞	2.5(0.7)	∞	0.11(0.03)	∞
6C	50.3	5.5(1.2)	-	1.3(0.2)	-	5.9(1.2)	-	1.4(0.3)	-	11.1(2.8)	-	7.4(1.8)	-	4.8(0.6)	-	0.21(0.04)	-
6M	49	3.4(1.2)	1	0.7(0.1)	∞	3.4(1.1)	∞	0.7(0.1)	∞	4.3(1.8)	1	17.2(3.0)	∞	3.1(0.6)	∞	0.13(0.03)	∞
7C	68	7.0(3.6)	-	1.2(0.2)	-	6.9(2.9)	-	1.2(0.1)	-	14.1(4.1)	-	8.7(2.3)	-	4.2(0.6)	-	0.21(0.06)	-
7M	64.8	3.3(1.4)	110	0.7(0.1)	∞	3.3(1.6)	18	0.7(0.2)	∞	4.4(1.9)	∞	17.2(3.4)	∞	2.8(0.4)	∞	0.11(0.03)	∞
8C	59.2	3.8(1.9)	-	1.3(0.2)	-	4.4(2.0)	-	1.6(0.3)	-	13.0(8.4)	-	36.2(7.6)	-	4.3(1.0)	-	0.20(0.04)	-
8M ⁹	58	2.7(1.5)	5×10 ⁴	0.6(0.2)	∞	2.6(1.7)	35×10 ²	0.6(0.2)	∞	7.5(6.7)	2.7×10 ⁴	45.6(6.2)	1.3×10 ²	1.5(0.8)	∞	0.08(0.04)	∞
9C	52.3	8.1(2.3)	-	1.5(0.3)	-	10.1(3.3)	-	1.9(0.4)	-	38.9(9.5)	-	8.4(3.2)	-	4.9(0.8)	-	0.20(0.04)	-
9M	49.8	4.2(1.5)	∞	0.7(0.2)	∞	5.4(1.1)	∞	0.9(0.1)	∞	23.3(11.3)	30	22.2(5.6)	∞	1.8(0.6)	∞	0.08(0.03)	∞
10C	52.8	6.7(3.3)	-	1.3(0.3)	-	7.6(3.6)	-	1.5(0.3)	-	14.5(3.9)	-	8.4(2.4)	-	4.5(0.6)	-	0.22(0.06)	-
10M	51.8	3.5(1.2)	230	0.8(0.1)	∞	4.0(1.6)	2.8×10 ²	0.9(0.1)	∞	5.3(1.6)	∞	20.4(3.5)	∞	2.8(0.4)	∞	0.14(0.03)	∞
11C	60.1	6.1(2.0)	-	1.4(0.3)	-	7.0(2.2)	-	1.6(0.4)	-	19.4(15.5)	-	7.7(2.6)	-	5.0(0.8)	-	0.21(0.04)	-
11M	52.3	3.7(0.5)	15	0.7(0.1)	∞	4.1(0.7)	5	0.8(0.1)	∞	5.4(1.7)	280	17.8(3.9)	∞	2.5(0.8)	∞	0.13(0.04)	∞
Contr. Mean		6.4(1.4)		1.3(0.2)		7.0(1.7)		1.5(0.2)		18.0(7.9)		10.6(8.5)		4.5(0.4)		0.21(0.01)	

Case No.	Age (yrs)	Chr.1 ²	P ³ ×10 ⁻⁶	Cen 2 Ratio ⁴	P × 10 ⁻⁶	Chrom 21	P × 10 ⁻⁶	Cen2 Ratio	P × 10 ⁻⁶	Inter-phase	P × 10 ⁻⁶	MNCANS ⁵	P × 10 ⁻⁶	Chr. 1 tel.length in μm	P × 10 ⁻⁶	Ratio ⁶	P × 10 ⁻⁶
MCI Mean		3.2(0.5)		0.7(0.1)		3.4(0.9)		0.7(0.2)		8.3(5.8)		24.1(9.3)		2.4(0.6)		0.15(0.01)	
Criterion		4.0		0.9		5.6		1.0		10.0		12.0		3.5		0.17	
Sensitiv.		1		1		1		1		0.82		1		1		1	
Specific.		0.91		1		0.82		1		1		0.91		1		1	
t(20)		7.26	1	10.47		6.55	2	8.62	∞	3.30	3,587	-3.55 ⁹	2,014	10.54	∞	15.77	∞

¹ cases 1-7 were female; Cases 8-11 were male;

² mean light intensity units ×10³ (Standard Deviation) from fluorescence emitted by a peptide nucleic acid (PNA) telomere probe labeled with FITC (fluorescein isothiocyanate) measured by a MetaSystems image analyzer; mean light intensity (and standard deviation) for chromosome 1 telomeres measured from 20 cells in metaphase;

³ P values were usually < .000000 or 10⁻⁶ abbreviated ∞; P value for 1(C) compared to 1(M) for chromosome 1 is .00014 (or 140×10⁻⁶) while for 2C versus 2M for Chromosome 1, the P value was .000000, indicated by ∞;

⁴ Ratio of light intensity from chromosome 1 telomeres / light intensity emitted by a PNA probe for Centromere 2;

⁵ MNCANS: mean number of chromosome arms with no signal;

⁶ Ratio of chromosome 1 telomere length in μm / (chr. 1 length - chr. 1 telomere length)

⁷ Control: age- and sex-matched individual with Down syndrome but no MCI or dementia;

⁸ Mild Cognitive Impairment;

⁹ For study 8, a different label (Cy3) was used, producing a surprisingly high level of signal loss; because these two data points may represent outliers, the analysis was repeated with their omission. t(18) = 6.94, p<.0001; Mann-Whitney U = 0, p<.0001.