Association of telomere length with cognitive impairments

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Abstract

Telomere attrition is attributed to Alzheimer's disease (AD), major depressive disorder, stress levels, physical inactivity, short sleep duration, and reduced educational abilities. In this article, we tried to assess the association between the telomere length in peripheral blood leukocytes and level of cognitive impairment and its dependence on age and sex.

Healthy subjects and patients with amnestic mild cognitive impairment (aMCI) and different AD stages were recruited in the study. All patients were assessed by the same standard diagnostic procedure, including neurological examination – Mini-Mental State Examination (MMSE). Blood samples from 66 subjects (18 men and 48 women, mean age 71.2 ± 0.56 years) were collected for DNA extraction from peripheral mononuclear cells (PBMC). Relative telomere length (RTL) was measured by monochrome multiplex polymerase chain reaction.

Obtained in the study data indicate that RTL in PBMCs has a statistically significant association with MMSE score ($p<0.02$). Moreover, the sex-specific difference was observed for the association between telomere length and various parameters of MMSE. Also, it has been found that a decrease in RTL by one unit is associated with an increase in the odds to get AD with the odds ratio of 2.54 (95% CI, 1.25 to 5.17).

The results obtained in this research are in coherence with other studies that telomere length may be a valuable biomarker of cognitive decline. However, the potential need for longitudinal studies of telomere length to estimate the influence of hereditary and environmental factors remains.

Introduction

Telomeres are the special nucleoprotein/DNA-protein complexes, represented as conservative, repeated, G-enriched sequences that vary among different species (Hastings et al. 2017). They are located at the ends of chromosomes and play a major role in genome protection either by preventing nuclease activity and chromosomal fusion or preventing any unwanted actions, as, for example, recombination (Blackburn 1991). After each somatic cell, replication cycle telomeres are shortened by approximately 30–200 nucleotides because of the incomplete replication of the DNA lagging strand. Such shortening is known to play a meaningful role in replicative senescence, inducing a cell cycle arrest. Also, it could lead to the anti-neoplastic effect and serves as a sign of cell senescence.

An enzyme responsible for telomeres elongation is called telomerase – a reverse transcriptase that has its own RNA template. It averts the telomeric loss which occurs due to incomplete replication of the lagging strand and exposure to oxidative stress (Blasco 2005). Telomerase activity is required for telomere length maintenance in embryonic, germline, and most stem cells, as well as actively proliferating somatic cells and malignant cells. It should be mentioned, that activated telomerase is associated with cellular immortality, and it may play a key role in the development of cancer (Zglinicki et al. 2000). TERT (catalytic subunit of telomerase) is detectable in tissues that are actively proliferating and exhibits high activity in embryonic and fetal cells, as well as in most somatic tissues, except the brain (Wright et al.

1996). At low levels, telomerase activity is detectable in normal human T and B cells: it usually increases in hematopoietic progenitor cells upon their proliferation and differentiation, and decreases with aging (Blasco 2005). However, the most active telomerase is in immortal human cells, as was noticed in most types of cancer. Among the ways for protection of telomeres should be noted an alternative lengthening of telomeres based either on the homologous recombination between telomeric/subtelomeric sequences or on the usage of extrachromosomal telomeres repeats (Dunham et al. 2000).

The main reasons for telomere shortening are the lagging strand end-replication problem, oxidative stress, chronic inflammation, failure of telomere's reparation, and various environmental factors. The primary size of telomeres is considered partially heritable and reduces with replication of somatic cells (Bischoff et al. 2005). Environmental agents also adversely affect such erosion along with cell division. Specifically, long-term chronic inflammation and oxidative stress were shown to promote telomere attrition (Demissie et al. 2006). Telomeres shortening in inflammation is associated with higher leukocyte proliferation and increased secretion of pro-inflammatory cytokines (Valdes et al. 2007). Additionally, oxidative stress decreases telomerase activity causing telomeres replication defects and triggering telomeres reduction (Boccardi et al. 2020).

Oxidative stress may be induced by cigarette smoking leading to telomere erosion and worse cognitive performance in senior ages (Stewart et al. 2006). Alcohol consumption, obesity, and diabetes mellitus probably may also contribute to changes in telomere length and cognitive capability. In recent times, the interaction of genetic, psychosocial and environmental factors is believed to affect telomere length and aging-associated diseases (Rask et al. 2016).

Since their discovering telomeres are a great point of interest for many scientists for several reasons, one of which is the possibility of understanding and predicting essential characteristics of tissues and cells based on their telomere length. This leads to further speculations on whether telomere length can be used as a biomarker of predisposition to some pathologies and if the rate of telomeres shortening is a factor that should be attributed to cognitive decline prediction. Although, some researchers found almost no to little association of telomere length with age-related diseases and cognitive decline (Mahoney et al. 2019; Movérare-Skrtic et al. 2012), another explicit connection between them (Koh et al. 2020; Rask et al. 2016). In general, studies on interactions between age-related diseases, cognitive decline, and telomere length have yielded inconsistent results, indicating a need for further investigations in order to understand deeper the processes that drive cognitive decline and telomere shortening.

The association between telomere length and cognitive functions also may be explained by their interaction with oxidative stress (Barnham et al. 2004) and inflammation (Yaffe et al. 2003). Both inflammation and oxidative stress are the major causes, following genetics, of the neurodegeneration and cognitive decline in the older adults (Barnham et al. 2004; Demissie et al. 2006; Yaffe et al. 2003).

Several studies are explicit that critically short telomeres are related to aging-associated chronic diseases and aging (Sanders and Newman 2013). Telomere attrition is attributed to major depressive disorder, stress levels, physical inactivity, short sleep duration, and reduced educational abilities (Starkweather et

al. 2014). Thus, telomere length can be a prospective marker of aging (Simm et al. 2008) and is relevant to the lifespan, cancer (Jang et al. 2008), Alzheimer's disease (AD) (Panossian et al. 2003), cardiovascular disease (Fitzpatrick et al. 2007), and osteoporosis (Valdes et al. 2007).

In this article, we tried to establish the association between the telomere length in peripheral blood leukocytes and the level of cognitive impairment, and its dependence on age and sex.

Methods

Selection of patients

Healthy subjects, and the patients with amnestic mild cognitive impairment (aMCI) and different stages of AD were recruited from the neurological department of the State Institution "D. F. Chebotarev Institute of Gerontology of the National Academy of Medical Sciences of Ukraine". All recruited participants were the residents of the urban areas of Kyiv region.

All patients were assessed by the same standard diagnostic procedure, including neurological examination – Mini-Mental State Examination (MMSE). The level of cognitive impairment was assessed with the Petersen criteria (Petersen 2012). In addition, all patients had undergone magnetic resonance imaging, urine and blood tests. Subsequently, those who had other mental disorders, signs of inflammation or metabolic decompensation were excluded. Blood samples from 66 subjects (18 men and 48 women, mean age 71.2 ± 0.56 years) were chosen for the further experiment. They were divided into a control group (18 healthy subjects, with a mean age of 69.9 ± 1 years) and a group with cognitive impairments (15 men and 33 women of 71.6 ± 0.65 years). According to our preliminary assessments, gender-related differences did not influence significantly, so we performed our final calculations combining women and men, thus increasing the statistical power of the study.

Ethical aspects

Ethics committee of the State Institution "D. F. Chebotarev Institute of Gerontology of NAMS of Ukraine" approved the study protocol. All participants gave written informed consent. In addition, the Helsinki Declaration (2000) and applicable national standards regarding their participation in the research were taken into account.

Measurement of leukocyte telomere length

Blood collection was performed during clinical examination in vacutainers containing EDTA (4 ml). After that, peripheral mononuclear cells (PBMC) were isolated from the blood on a continuous Biocoll gradient (1.077 g/ml, Biochrom, Germany) following centrifugation (400×G, 30 min, at room temperature). Then the interphase containing PBMC peripheral blood monocytes with a part of an upper plasma phase was taken and washed with 50 ml of phosphate buffer saline. After centrifugation (250×G, 6 min) cells were frozen, and stored at -80°C until further usage. DNA extraction was performed using the phenol-chloroform method (Köchl et al. 2005).

Relative telomere length (RTL) was measured by monochrome multiplex polymerase chain reaction, originally described by Cawthon (Cawthon 2009). Reaction master mix preparation included the following reagents: commercial reagent kit for RT-PCR (2.5x Reaction mix for the RT-PCR with SYBR Green I, Syntol), betaine (at 1M concentration, Sigma-Aldrich) and pairs of primers (*telg* and *telc* – 450 nM, *albu* and *albd* 250 nM). All samples were tested in triplets, and for the calibration curve, four serial dilutions varying from 1 to 1/27th were made. The thermal cycling profile was as follows: 95°C for 15 minutes; 2 cycles: 94°C – 15 s and 49°C – 15 s; 32 cycles: 94°C – 15 s, 62°C – 10 s, 74°C – 15 s and signal acquisition, 84°C – 10 s, 88°C – 15 s – and signal acquisition. As software for amplification curves generation was chosen Opticon Monitor 3. Telomere DNA quantity to single-copy gene DNA quantity ratio then was used to calculate an average RTL (T/S ratio).

Statistical analysis

Based on the Shapiro-Wilk test for normality distribution, non-parametric tests were used for the statistical analysis. First, the equality of males and females proportions in groups was assessed with Fisher's exact test. Second, the significance for the association of RTL with MMSE score was tested by the Jonckheere-Terpstra (JT) trend test. Third, a linear regression was built for RTL and MMSE. Fourth, correlations assessment was performed by Spearman's test. Finally, an ordinal logistic regression model was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between RTL (predictor) and MMSE group (outcome). Analyses were performed by Statistica 8.0 (StatSoft Inc.) and SPSS Statistics (v. 26, IBM Corporation) software.

Results

Characteristics of participants

Participants were divided according to the generally accepted MMSE results interpretation of 24–27 for the amnestic mild cognitive impairment (Folstein et al. 1975), MMSE ≤ 24 – for AD, and MMSE > 27 for patients without any cognitive impairments (a control group). MMSE consists of 11 primary subtests: temporal orientation (M1), spatial orientation (M2), immediate memory (M3), attention/concentration (M4), delayed recall (M5), naming (M6), verbal repetition (M7), verbal comprehension (M8), writing (M9), reading a sentence (M10) and constructional praxis (M11). Each subtest assigned the letter M and a number from 1 to 11, respectively.

The non-parametric tests have been used (Fig. 1). The proportions of males and females in all three groups were equally distributed. Essential characteristics of the studied cohort are represented in the Table 1, where may be observed no difference in age among the patients of three MMSE groups. At the same time, the statistically significant difference in T/S ratios has been found for all MMSE groups (p = 0.019). Specifically, after the test for pairwise comparisons statistically significant difference in T/S ratios has been found between patients with AD and controls p = 0.006.

Table 1
Characteristics of the participants grouped by MMSE score; **Jonckheere-Terpstra trend test**

	All (n = 66)	AD (n = 40)	aMCI (n = 8)	Control (n = 18)	*P*-value
m/f	18/48	12/28	3/5	3/15	0.193
Age [a]	71 (68–74)	71 (68–75)	72 (71–75)	69 (67–74)	0.863
MMSE [a]	21.5 (17–29)	19 (13–21)	25 (24.5–26)	30 (29–30)	**< 0.001**
T/S ratio [a]	1.22 (0.96–1.84)	1.16 (0.87–1.66)	1.42 (0.81–2.13)	1.68 (1.22–2.27)	**0.019**

Telomere length associations with cognition

According to Spearman's test, telomere length is positively correlated with MMSE score, as well as with some of its components M2, M4, M5, M7, M11 (Table 2).

Table 2
Correlations of cognitive parameters with relative telomere length for all study participants; **Spearman's test**

Parameter	M2	M4	M5	M7	M11	MMSE
Correlation Coefficient	0.347	0.314	0.36	**0.455**	0.329	**0.34**
P-value	0.02	0.036	0.015	**0.002**	0.027	**0.005**

Gender-related differences have been found in correlations between MMSE parameters and relative telomere length. Specifically, in males, the only association of T/S with M11 has been identified (Table 3).

Table 3
Correlations of cognitive parameters with relative telomere length for males and females separately; **Spearman's test**

Parameter		M1	M2	M4	M5	M7	M11	MMSE
Males	Correlation Coefficient	-0.046	-0.003	0.139	0.307	0.146	**0.761**	0.322
	P-value	0.881	0.991	0.649	0.307	0.633	**0.003**	0.192
Females	Correlation Coefficient	**0.416**	**0.524**	**0.447**	**0.396**	**0.628**	0.172	**0.372**
	P-value	**0.018**	**0.002**	**0.01**	**0.025**	**0.000**	0.347	**0.009**

On the contrary, in females, the positively directed association of T/S is observed for M1, M2, M4, M5, M7, M11 and MMSE. However, it should be noted that these gender-related differences could have arisen because of a smaller number of males compared to females among participants.

Associations of telomere length with MMSE score are given in the Fig. 2 as a regression model. A significant association has been found between RTL and MMSE score: $R^2 = 0.1104$, $p = 0.006$.

According to the ordinal regression analysis, a decrease in relative telomere length by one unit is associated with an increase in the odds to get Alzheimer's disease with the odds ratio of 2.54 (95% CI, 1.25 to 5.17), Wald $\chi^2(1) = 6.67$, $p = 0.01$.

Discussion

Telomeres are considered to be linked to lifespan (Simm et al. 2008). The main reasons for their potential application as biomarkers of aging are shortening with each cell division and higher susceptibility of telomeres to oxidative stress compared to the rest of genomic DNA. An increased accumulation of DNA damage in telomeres is based on the properties of TRF2 (Telomeric repeat-binding factor 2) to inhibit the non-homologous end-joining. In addition, telomeres are more likely to get 8-oxoG mutations because of the generally large quantity of guanidine bases, as well as to get single-strand breaks. Recent studies have agreed that telomeres are rather markers of damage repair capacity of a cell (Boonekamp et al. 2013) and of an oxidative stress level (Houbern et al. 2008). Considering that age can be characterized by general cognitive functions and telomeres length, these factors are also expected to be interconnected.

This study did not find any associations between age and telomere length, compared to other studies (Müezzinler et al. 2013), but this can be explained by the narrow range of patients' age. However, age have shown a positive correlation with spatial orientation. In terms of the difference in mean RTL of patients with Alzheimer's disease, those with aMCI and control group, our results are consistent with results of other studies (Honig et al. 2006; Valdes et al. 2010). The finding may be supported by a recent study that shows a high chance of MCI development in patients with both short and long telomeres (Roberts et al. 2014). We concluded that the mean RTL of patients with AD is significantly lower than in those from the control group. However, we could not assert about statistically significant differences in T/S ratios between aMCI and control groups, though mean RTL in subjects with aMCI was lower than in the control group.

Regarding gender-related distinctions, our results were similar to those of other researchers (Hochstrasser et al. 2012), as gender-related interactions did not show any statistically significant impact on RTL in our study. However, at the same time, some studies demonstrated longer telomeres in women compared to men (Barrett and Richardson 2011).

Our findings suggest that there is a strong association of RTL with MMSE score. Specifically, we observed the correlation between the T/S ratio and specific cognitive processes, including focused and sustained attention, verbal fluency, and constructive praxis. The mechanisms of such dependence may be due to increased oxidative stress (Sultana et al. 2009) or inflammation (Yaffe et al. 2003). Previous studies showed the interconnection of oxidative stress (Demissie et al. 2006) and inflammatory markers (Fitzpatrick et al. 2007) with telomere length. These factors are involved in neurodegenerative disorders

progression (Barnham et al. 2004; Yaffe et al. 2003). Moreover, genetic factors also affect telomere length and cognitive aging as described by Andrew et al. (Andrew et al. 2006). Twin and family surveys proved the heritability of telomere length, which is approximately 36%.

On the other hand, short telomeres in neurons, astrocytes, and immune cells may increase the proinflammatory mediators secretion and oxidative stress-induced senescence, consequently triggering the disease development (Jurk et al. 2012; Weng 2012). The shortest telomeres inside a cell were shown to have the most striking effect on cell phenotype and aging (Hemann et al. 2001). Telomere length erosion indicates immune cell "depletion", and symptoms that occur in neurodegenerative diseases emerge due to the inability of the immune system to fight pathology (Schwartz and Shechter 2010). However, the evidence supporting that immune senescence caused by shorter telomeres is among the reasons why age is a primary risk factor for several neurodegenerative diseases is sparse (Eitan et al. 2014). Thus, the telomere length is vital for cellular endurance maintenance in the elderly older adults and for the processes of aging, such as deterioration of cognitive abilities (Blackburn et al. 2015). That fact was proven on mice models with telomerase deficiency, which leads to the elevated aging speed with improper tissue repair and disrupted neurological functions, and with subsequent functions restoration after telomerase reactivation (Jaskelioff et al. 2011). However, findings in the field of oxidative stress suggest that TERT, which preserves telomeres from shortening in normal conditions, could have another non-canonical oxidative stress prevention function. Under oxidative stress, telomeres deteriorate at higher rates, as telomerase is excluded from the nucleus and translocated to mitochondria. Therefore, the functions of telomerase outside the nucleus should be investigated. In cells with enhanced TERT expression, mitochondrial performance is amended, mitochondrial DNA (mtDNA) is protected, and cell peroxide and mitochondrial superoxide levels are reduced, leading to improved stress resistance and antioxidant defense. Specifically, in neurons, TERT demonstrates a protective effect against tau- and amyloid-mediated oxidative stress and apoptosis (Spilsbury et al. 2015). In addition, longer telomeres enhance TERT up-regulation that is advantageous during some diseases and inflammation. Mitochondria mediated stress and their disfunction significantly impact neurodegeneration and neuron death. As the TERT level, the efficiency of the antioxidant system and mitochondrial disfunction have strong inheritable components and are shared by all organism cells – RTL could be a relevant marker of neuronal state, as they are affected by all of the mentioned systems.

Nonetheless, whether telomere shortening is a cause or an outcome of different diseases is still under debate (Kordinas et al. 2016). Furthermore, pathological processes also may be responsible for the attrition of telomeres, while short telomeres may cause a pathological state via oxidative stress and inflammation as well. Thus, the limitations of the individual approach to the research must be considered when interpreting the data, and it should be noted that RTL as a biomarker alone can give insight into the ongoing disease (Levstek et al. 2020).

At the same time, there are some limitations of our study. First, the sample size was relatively small, and a more significant number of patients is required to designate the complex interactions between pathology, age, telomeres and their contribution to cognitive impairment and dementia. Secondly,

telomere length measured in PBMC probably may not be a perfect representative of telomere length in brain cells. Even though the direct relationship between the length of telomeres in the brain and peripheral blood cells was reported (Lukens et al. 2009), leukocytes are known to express telomerase and thus preserve the telomere length during proliferation (Akbar and Vukmanovic-Stejic 2007). Furthermore, it is still unclear whether the telomere shortening in leukocytes corresponds to such in neurons or not because the reduced level/absence of cell turnover may indicate the absence of telomere attrition in healthy brain (Nakamura et al. 2007). The conflicting outcomes were presented in investigations of telomere length in the brain of patients with AD (Lukens et al. 2009). Consequently, the question of whether the leukocyte telomere length is a marker of the telomere length in brain tissues remained unanswered. The peripheral immune status activation, possibly associated with brain inflammation, was noticed in AD and MCI (Liu and Chan 2014). In this regard, it can be assumed that the shortening of the telomeres in leukocytes may be an indicator of that phenomenon, which goes along with the progression of the disease from prodromal conditions (aMCI) to the full development of AD. However, further studies should be conducted to elucidate the specific role of telomere erosion in the pathogenesis of AD.

Together with some other studies, our research suggests that telomere length may be a valuable biomarker of cognitive decline and inflammatory diseases in general. However, such biomarkers can be implemented only after longitudinal studies in various cohorts, with appropriate adjustment for relevant factors. Moreover, there is a need for a generalized and standardized method of telomere length measurement that includes all stages of an experiment: from taking samples to interpreting results.

To sum up, we observed significantly lower telomere length in patients with AD compared to the control group, and such data indicate that RTL in PBMC has a strong association with MMSE score. However, there are limitations to the mentioned dependence because of the general telomere length variability between different individuals. Thus, there is a potential need for longitudinal studies of telomere length to estimate the influence of hereditary and environmental factors.

Declarations

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Consent to participate All participants gave written informed consent.

Conflict of interest The authors declare no competing interests.

Authors' contributions

Diukov Y.: design of the experiments, selection of patients, samples' preparation;

Bachinskaya N.: design and coordination of the experiments;

Barsukov O.: introduction, editing of the manuscript;

Dziobak A.: PCR analysis, writing the draft, support data analysis and interpretation of the results;

Kholin V.: conceptualization, investigation;

Kyriachenko Y.: PCR analysis, writing the draft, support data analysis and interpretation of the results;

Zabuga O.: statistical analysis, editing of the manuscript, formatting and final preparation;

Krasnienkov D.: conceptualization, investigation, editing of the manuscript, discussion and interpretation of the results.

Ethics approval Ethics committee of the State Institution "D. F. Chebotarev Institute of Gerontology of NAMS of Ukraine" approved the study protocol. The Helsinki Declaration (2000) and applicable national standards were taken into account.

Data availability Data generated in the present study are available from the corresponding author on reasonable request.

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Figures

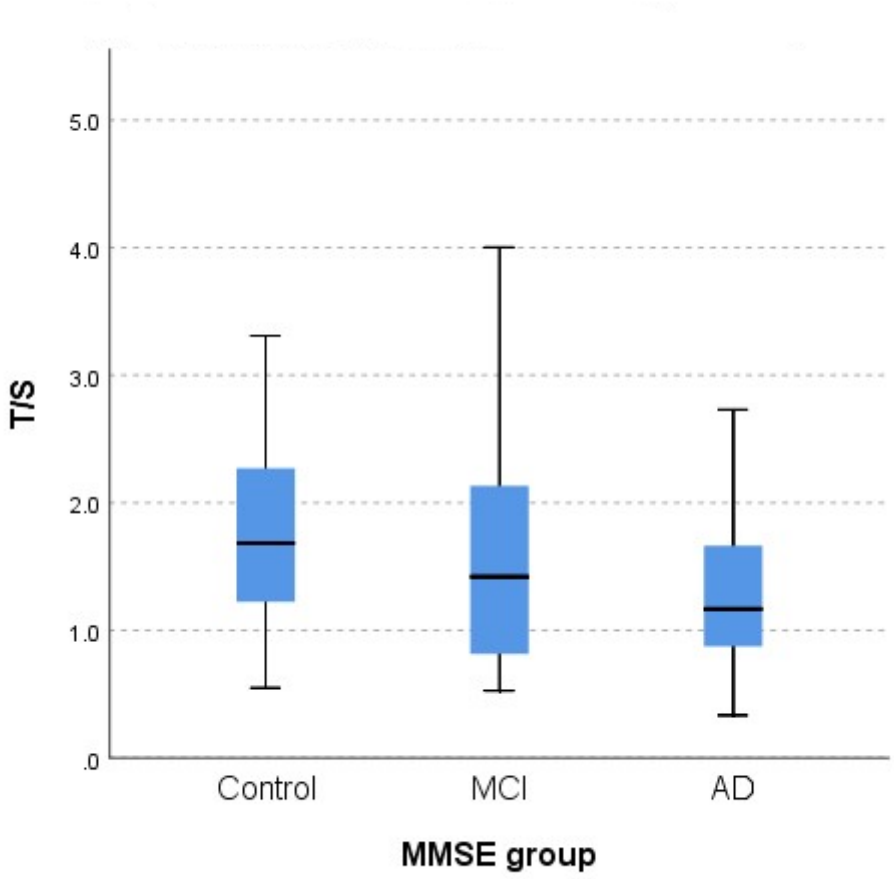

Figure 1

The box-and-whisker plots for relative telomere length in three MMSE groups; Jonckheere-Terpstra trend test

Note. MMSE – Mini-Mental State Examination; AD – Alzheimer's disease; aMCI – amnestic mild cognitive impairments; T/S – telomere DNA quantity to single-copy gene DNA quantity ratio.

In each box-and-whisker plot, the box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line inside the box represents the median. The whiskers above and below the box show the locations of the minimum and maximum values.

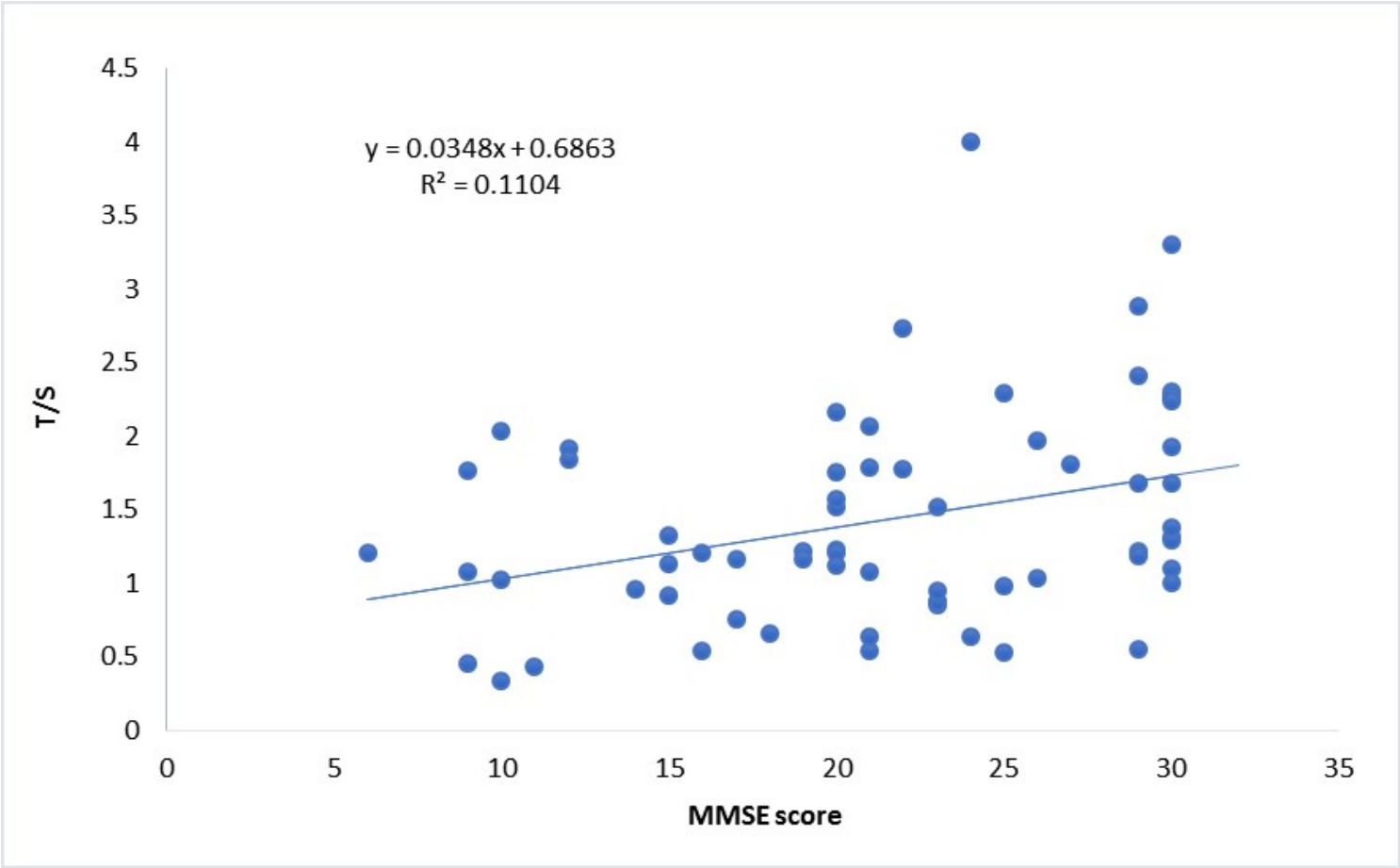

Figure 2

Relationship scatterplot for relative telomere length and MMSE score; Univariate linear regression

Note. MMSE – Mini-Mental State Examination; T/S ratio – telomere DNA quantity to single-copy gene DNA quantity ratio.