Psychological states and physical fates

van Ockenburg, Sonja

Chapter 4

Does neuroticism make you old? Prospective associations between neuroticism and leucocyte telomere length

Sonja L. van Ockenburg, Lineke M. Tak, Stephan J.L. Bakker, Reinold O.B. Gans, Peter de Jonge, Judith G.M. Rosmalen

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ABSTRACT

Background

Telomere attrition, causing accelerated aging might be one of the mechanisms through which neuroticism leads to somatic disease and increased all-cause mortality. In the current study we investigated whether neuroticism is prospectively associated with shorter telomere length (TL), a biological marker of aging.

Methods

Participants were 3432 adults (mean age 52.9 years, minimum-maximum 32-79). Data was collected at baseline (T1), and at two follow-up visits after 4 years (T2) and 6 years (T3). Neuroticism was assessed through the 12-item neuroticism scale of the Eysenck Personality Questionnaire-Revised at T2 and T3. TL was measured by monochrome multiplex quantitative PCR at T1, T2, and T3. A linear mixed model was used to assess if neuroticism could predict TL prospectively after adjusting for age, sex, BMI, frequency of sports, smoking status, presence of chronic diseases, and level of education.

Results

Neuroticism was a significant negative predictor of TL at follow-up ($B = -.004$; $p = .044$) after adjusting for sex, age, baseline telomere length, and various biological and lifestyle factors.

Conclusions

High neuroticism is significantly and prospectively associated with telomere attrition independent of lifestyle and other risk factors.

INTRODUCTION

Neuroticism is considered one of the personality traits most important to public health due to its association with, and its ability to predict various mental [1] and physical disorders [2], including cardiovascular disease [3]. Neuroticism measures refer to individual differences in the tendency to experience negative emotions, especially when confronted with threat, frustration or loss [4]. Operationally, neuroticism is defined by items referring to negative affect, such as anxiety, irritability, anger, worry, self-consciousness, frustration, reactivity, vulnerability, hostility, sensitivity to criticism of others, and accompanying behavioural and cognitive traits [5]. It has, therefore, been suggested that neuroticism is a measure for a person's set point of negative affect [1]. Moreover, neuroticism scores prospectively predict person-dependent stressful life events (i.e. adversities that a persons might have brought upon themselves) and chronic adversity [6].

Several studies have evaluated the potential role of the hypothalamic pituitary adrenal axis (HPA axis) in explaining the association of neuroticism with adverse health outcomes. Unfortunately, results of studies investigating the relationship between neuroticism and the HPA axis are inconsistent, with studies reporting either positive [7, 8], negative [9, 10] or no relationship [11-13]. Therefore the mechanisms by which neuroticism may affect somatic health have yet to be elucidated. An interesting new perspective is offered by recent findings that psychosocial stress (e.g. childhood adversities, caregivers stress) is associated with shorter telomere length both cross-sectionally [14-16] and prospectively [17, 18], as shortening of telomeres is related to the process of cellular aging [19]. Moreover, a large cross-sectional study investigating the relationship between trait hostility and telomere length found telomeres to be significantly shorter in high-hostile men [20]. Telomeres are specialized chromatine structures that "cap" the ends of chromosomes in eukaryotic cells. Telomeres prevent chromosome ends from being recognized as double stranded DNA breaks, promoting chromosomal stability [21]. In addition, they play an important role in regulating the replicative lifespan of cells [22] and in stem cell mobility [23]. DNA-polymerases cannot copy the end of chromosomes and a special enzyme called telomerase is needed to add telomere repeats during cell division. However, in somatic cells, only limited amounts of telomerase are present, thus telomeres shorten progressively with each cell division [24]. Short telomeres are predictive of increased mortality rates [25, 26] and increased incidence of various age-related diseases, such as cancer [27], and Alzheimer's disease[25].

As various forms of psychosocial stress are associated with shorter telomeres [14-16, 18, 28] and neuroticism scores prospectively predict exposure to person-dependent stressful life events and chronic adversity [6], accelerated telomere shortening might be one of the mechanisms through which neuroticism leads to somatic disease. A longitudinal study might be able to provide insight into the sequence of events and make more accurate statements about the direction of causality.

The aim of the present study is to prospectively test the effect of neuroticism on telomere length in a large population based cohort. We hypothesize that higher scores of neuroticism are associated with shorter telomere length as neuroticism is a predictor of a person's habitual level of distress, exposure to stressful life-events, and interpersonal difficulties. Up

to now this hypothesis remains untested. If it were to be true, it could mean an important step forward in our understanding of the relationship between neuroticism and somatic disease and longevity.

METHODS AND MATERIALS

Study population

Our study has been performed in a cohort derived from Prevention of REnal and Vascular ENd stage Disease (PREVEND), a population cohort study originally designed to investigate microalbuminuria as a risk factor for renal and cardiovascular disease. The recruitment of participants for PREVEND has been extensively described elsewhere [29]. In brief, all inhabitants of the city of Groningen between the ages of 28 and 75 years (85,421 subjects) were asked to send in a morning urine sample and to fill out a short questionnaire on demographics and cardiovascular history. A total of 40,856 subjects (47.8%) responded. After exclusion of subjects with insulin dependent diabetes mellitus and pregnant women, all subjects with an elevated urinary albumin concentration (UAC) of ≥10 mg/l (N = 7,768) together with a randomly selected control group with a UAC of <10 mg/l (N = 3,395) were invited for further investigations (total N = 11,163). Finally, 8,592 subjects completed the total screening program, rendering the PREVEND study cohort. The PREVEND study is enriched for albuminuria which is a risk factor for developing renal disease. We, however, wanted to study a cohort that is a representative sample of the Groningen population and not at heightened risk for specific diseases. To that purpose we took all subjects with a UAC <10 mg/L that completed the first screening (N=2592) and added a subset of the "oversampled" subjects with an UAC >10mg/L by proportionally taking a SPSS generated random subset (n=840). This resulted in a group of 3432 subjects with a population representative ratio of albuminuria negative and positive subjects forming the basis for the current study. Three waves of data were available for this study: The baseline screening was completed in 1998 (T1), followed by two follow-up visits at 4.2 (T2) and 6.4 (T3) years from baseline. The study was approved by the local Medical Ethical Committee for human research of the University Medical Center Groningen (UMCG). All participants were aged 18 or older and provided written informed consent for participation in this study.

Neuroticism

Participants completed the Dutch translation of the 12-item neuroticism scale of the Eysenck Personality Questionnaire-Revised (EPQ-RSS-N)[30] at home prior to their visit of our research facilities at T2 and T3. The EPQ-RSS-N comprises 12 questions, representing nervousness, emotional lability, feelings of guilt, and low self-esteem, in a "yes"/"no" format. For each participant, a sum score was constructed by adding the questions answered in the affirmative. The sum score, therefore, represents the total number of neuroticism symptoms reported. Missing data were imputed according to the method of corrected item mean substitution, if at least half of the items were completed [31]. For the EPQ-RSS-N sum score, of the 135 participants who had at least one missing item, 12 were imputed, resulting in 2721 valid EPQ-RSSN sum scores (95.7% of the study sample at T2). The EPQ-RSS-N exceeded the

criterion for acceptable instrument internal consistency reliability of 0.70 or greater [32]. The psychometric characteristics of the EPQ-RSS-N were as follows: Crohnbach's α=.86; mean inter-item correlation, 0.35; range of item-rest correlations, 0.43-0.64. Test-retest coefficient for EPQ-RSS-N sum score in this population was 0.73, average test–retest interval=2.4 years).

Covariates

Covariates were selected for their known association with neuroticism or telomere length: BMI [33], smoking [33] (none, 1-5 cigarette(s)/day, 6-10 cigarettes/day, 11-15 cigarettes/day, 16-20 cigarettes/day, >20 cigarettes/day), frequency of sports [34] (I don't exercise, once per week, twice or more per week), presence of a chronic disease (Coronary heart disease (CHD), cerebrovascular accident (CVA), diabetes mellitus, chronic liver-disease, chronic kidney-disease, malignancy, rheumatoid arthritis, COPD or asthma, severe skin disease, severe bowel disease lasting > 3 months), and level of education [35] (low, middle, high). The somatic diseases, except for diabetes, CHD, and CVA were self-reported diseases that were present in the previous year. Diabetes was defined as the use of antidiabetic treatment according to self-report or pharmacy data. CHD and CVA were defined as self-report of CHD/CVA upon inclusion in the study and/or confirmed occurrence of CHD/CVA between inclusion and date of visit to the research facilities at T2. Low educational level was defined as lower secondary education or less, middle educational level was defined as higher secondary education, and high educational level was defined as tertiary education.

Telomere length

Fasting blood samples were collected from all participants by a nurse during a visit to the research facilities. In case of flu or a febrile temperature, blood collection was postponed to a later time. Telomere length in the PREVEND cohort was measured in leucocytes at T1, T2, and T3 by a monochrome multiplex quantitative PCR method, whereby telomere specific amplification and the reference gene amplification take place in a single reaction well [36]. All samples were measured in triplicate and the average of the three runs was used to provide the mean relative measure of telomere length for each individual. The mean telomere repeat sequence copy number (T) was compared to a reference single copy gene copy number (S) in each sample. T/S = 1 when the unknown DNA is identical to the reference DNA in its ratio of telomere repeat sequence copy number to single copy gene copy number. The calibrator sample used was made up of a mixture of DNAs from young adult individuals (around 25 years). The intra-assay coefficient of variation was 2% (T), 1.9% (S) and 4.5% (T/S ratio). Reproducibility data was obtained for 216 subjects from PREVEND and good agreement between T/S ratios was observed (R^2=0.99, P<0.0001, inter-run CV 3.9%). There was a highly significant decline in T/S ratio with age in PREVEND (-0.0047 (SE 0.0004) decrease in T/S ratio per year increase in age (P<.0001) confirming the internal validity of the assay. Telomere length at was available for 3209 participants at T1 and for 2298 at T3. Unfortunately, DNA and thus telomere length at T2 was only available for a subset of the population (N=1236)).

Statistical analyses

We used a linear mixed model to account for the non-independence of observations (repeated measurements were nested within individuals). The model contained telomere

length at T2 and T3 as the dependent variable and neuroticism (at T2 and T3) as a time varying predictor. The model included as covariates gender, BMI, smoking, frequency of sports, presence of a chronic disease, level of education, telomere length at baseline (T1), and time in years between measurement occasions. Below a composite specification of the model is given.

$$Telomere\ length_{ij} = \gamma_{00} + \gamma_{01}\ Telomere\_length_{i} + \gamma_{02} neuroticism_{ij} + \gamma_{03} AGE_{i} + \gamma_{04} Gender_{i} + \gamma_{05} Presence\ of\ a\ Chronic\ disease_{i} + \gamma_{06} Education_{i} + \gamma_{07} Smoking_{ij} + \gamma_{08} Sports_{ij} + \gamma_{09} BMI_{ij} + \gamma_{10} Time_{ij} + (\varepsilon_{ij} + \zeta 0_{i})$$

In this notation $Telomere\ length_{ij}$ denotes the length of the telomeres for individual *i* at measurement occasion *j*. γ_{02} denotes the average effect of the sum score of neuroticism symptoms at T2 and T3 on telomere length (T2, T3), adjusted for telomere length at T1. $\zeta 0_{i}$ denotes the random intercept variance. Maximum likelihood method was used for model estimation. The distribution of telomere length was checked for normality. As it had a slight positive skew, telomere length was naturally log transformed to meet the assumption. For each model it was checked if associations were linear, quadratic or cubic. Results were considered statistically significant for a two-sided P-value <0.05. All models were analyzed using the nlme package [37] in R, version 2.15.2 [38].

RESULTS

Study population

Descriptive statistics for our study population are provided in table 1. During the PREVEND study at T2, and at T3, 540 and 1028 participants respectively, did not show up at the follow-up visit. Some of the participants, however, that were not present at follow-up at T2, did show up for the follow-up visit at T3 and vice versa. A total of 572 (17%) did not show for any of the follow-up visits and had only baseline data available. From the above it becomes clear that as any longitudinal study PREVEND suffered from attrition. We therefore investigated the pattern of missingness. We assumed missing data at random (MAR) as missingness depended on the observed variables[39]. Attrition was related to heavier smoking, drinking more alcohol, having a higher BMI, exercising less, being lower educated, being more neurotic, and having shorter telomeres.

Previous longitudinal studies investigating telomere length have reported both the possibility of telomere attrition and telomere lengthening [40-43]. Most studies defined attrition as a decrease in telomere length>15% and lengthening as an increase in telomere length >15% between baseline and follow-up measures. We investigated the dynamics of telomere length in our cohort using these definitions. Over an average time of 6.5 years between baseline and follow-up 65.2% showed a decrease in telomere length, 6.9% remained stable, and 27.9% showed lengthening of telomeres. This shows that telomere length is highly dynamic.

Table 1. General characteristics of the study population

	T2
Gender (%)	
Male	48.1
Female	51.9
Age, mean (SD) min-max	52.9 (11.8) 32 - 79
Race (%)	
White	95.8
Black	0.8
Asian	1.9
Other	1.5
Neuroticism score, median (interquartile range)	2.0 (0.0 – 5)
Education (%)	
None	6.7
Low	30.2
Middle	27.0
High	36.2
Smoking yes (%)	25.4
Smoking cigarettes/day (%)	
none	74.6
1-5	4.5
6-10	5.1
11-15	6.9
16-20	6.0
> 20	2.8
Frequency of sports (%)	
Does not exercise	57.9
Once per week	23.7
Twice or more per week	18.4
BMI (kg/m^2) mean (SD)	26.5 (4.2)
Chronic diseases (%)	
healthy	80.9
1 chronic disease	15.5
2 chronic diseases	2.9
3 chronic diseases	0.5
4 chronic diseases	0.1
5 chronic diseases	0.1

Descriptive statistics for the population at T2.

Neuroticism and telomere length

The results of the fully adjusted analysis can be found in table 2. In a random intercept model adjusted only for gender, age, time, and baseline telomere length, the sum score of neuroticism symptoms predicted a significant decrease in telomere length (coefficient = −0.005; SE = 0.002*;* p = .008). In our second and final model, we added BMI, smoking, frequency of sports, the presence of chronic diseases, and education. The sum score of neuroticism symptoms remained a significant predictor of telomere attrition (coefficient = −0.004; SE = 0.002*;* p = .044). The coefficient of the sum score of neuroticism symptoms decreased only slightly, indicating that although lifestyle factors explain a portion of the variance, neuroticism also still explains a unique portion of the variance in

telomere attrition independent of life-style factors. Likewise, age was a significant predictor of telomere attrition. Furthermore, there was a non-significant trend of higher education being associated with telomere elongation. Surprisingly, smoking status, gender, and the presence of a chronic disease did not predict changes in telomere length.

Table 2. Mixed model predicting telomere length at T2 and T3 by the sum score of neuroticism symptoms, adjusting for baseline telomere length

N=2156	Coefficient	SE	p-value
Telomere length T1	0.184	0.022	<.001
Neuroticism	-0.004	0.002	.044
Age	-0.003	0.001	<.001
Gender (female)	0.016	0.012	.183
Precense of a chronic disease	-0.008	0.011	.490
Education			
low	0.013	0.025	.589
middle	0.024	0.026	.359
high	0.046	0.026	.073
Smoking	-0.007	0.005	.125
Sports			
once per week	-0.006	0.014	.695
twice or more per week	0.015	0.015	.330
Time	-0.004	0.005	.384

SE=standard error. Intercept and random intercept not shown.

DISCUSSION

To our knowledge, this is the first prospective large population based study showing that neuroticism is significantly associated with shorter telomere length over time, independent of BMI, frequency of sports, smoking, baseline telomere length, and level of education.

Neuroticism can be viewed as a person's habitual level of distress and predicts exposure to psychosocial stress, in particular stressful life-events and chronic difficulties [1]. Therefore, our results are in agreement with previous cross-sectional (Damjanovic et al., 2007, Epel et al., 2004, Kananen et al., 2010, Tyrka et al., 2010, Wikgren et al., 2012, Drury *et al* 2012) and prospective studies [17, 18] demonstrating an association between psychosocial stress and decreased telomere length. Also our results are in concordance with two cross-sectional studies linking personality traits, hostility [20] and pessimism [44], to shorter telomere length. As mentioned earlier, both neuroticism [3, 45] and telomere attrition [25, 26] are associated with increased all-cause mortality and various diseases of aging. Two mutually nonexclusive explanations for the entanglement of neuroticism, telomere length, and somatic diseases and increased mortality might be offered. One possibility is that telomere shortening is part

of the mechanism through which neuroticism leads to increased mortality rates, through for instance chromosomal instability [46] and cell senescence [22]. An alternative explanation, however, could be that for a large part, decreased telomere length, as well as increased mortality and neuroticism, are the result of exposure to other shared risk factors, such as the amount of lifetime oxidative stress exposure and genetic vulnerability for psychosocial stress. Glucocorticoids, stress hormones released from the adrenal gland under conditions of psychosocial stress [47], have been shown to increase damage by oxidative stress in neurons [48, 49]. Since high neuroticism scores are prospectively associated with exposure to more psychosocial stress [6], increased glucocorticoid exposure might be a mediating factor, explaining both telomere attrition and the increased morbidity and mortality that are associated with neuroticism. The results of a recently published study, showing that a hypocortisolemic state was associated with shorter telomere length in both patients with recurrent depression and healthy controls [50], contradicts the above stated hypothesis. We need to bear in mind, however, that this study was cross-sectional in nature, and therefore could not exclude that hypocortisolemia is the likely end result of a repeatedly overstressed and finally exhausted HPA-axis as suggested by Fries et. al. [51].

There are several strengths and limitations of the current study that need to be taken into consideration when interpreting our results. The first major strength of this study is that we conducted our study in a large population representative cohort increasing the generalizability of our findings. It needs to be mentioned, however, that our population consisted mainly of white people and the results of our study can thus not be generalized to people with other racial or ethnic backgrounds. The second strength is the prospective nature of the design, allowing us to model telomere attrition over time. This is the first prospective study demonstrating a significant relationship between a personality trait and telomere attrition. All other studies, up until now, have been cross-sectional, thus, not providing insight into the sequence of events [14, 20]. A first limitation of our study is that we measured telomere length by monochrome multiplex quantitative PCR. This makes it harder to compare our findings to that of other cohorts as the results of PCR are given in the form of a ratio and not in absolute kilobase pairs. It is, however, a commonly accepted and reliable method for measuring telomere length [36] and our assay has a good internal validity. A second limitation is that like any longitudinal study the PREVEND study suffered from attrition and no-show at some of the scheduled follow-up visits. The participants that fell out of the study or missed a visit had significantly shorter telomere length, significantly higher scores of neuroticism at baseline, and a significantly unhealthier lifestyle than participants that had not fallen out of the study. These attrition-associated differences will lead to either under or overestimation of the true effect of neuroticism on telomere length, as the most extreme cases for most variables have discontinued to participate in the study. Likelihood-based methods can, however, provide reliable estimates when the missing data at random assumption holds, as was the case in our study [52, 52]). Finally, the observational design of our study does not permit us to draw any conclusions about causality.

In conclusion, this is the first study demonstrating that neuroticism is prospectively associated with telomere attrition. Future studies could investigate whether increased glucocorticoid levels caused by repeated stress exposure is one of the mechanisms via which neuroticism exerts its negative effects on telomere length and physical and mental health.

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