Received Date : 09-Sep-2010
Revised Date : 24-Mar-2011
Accepted Date : 16-Jun-2011
Article type : Original Article

Article: Genetics

Leukocyte telomere length is associated with complications of Type 2 diabetes mellitus

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Running title: Telomere length and diabetic complications

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Abstract

Objective The key goal of diabetes management is to prevent complications. While the patho-physiological mechanisms responsible for diabetes complications have been extensively studied, at present it is impossible to predict which patient with diabetes could develop complications. In recent years, the role of leukocyte telomere length in the pathogenesis of cardiovascular disease and Type 2 diabetes has been investigated. However, studies aiming to investigate the role of telomeres in the development and progression of Type 2 diabetes, as well as diabetic complications, are still lacking. As a consequence, this study aimed to verify whether leukocyte telomere length is associated with the presence and the number of diabetic complications in a sample of patients with Type 2 diabetes.

Methods This is a cross-sectional study. Nine hundred and one subjects were enrolled, including 501 patients with Type 2 diabetes, of whom 284 had at least one complication and 217 were without complications, and 400 control subjects. Leukocyte telomere length was measured by quantitative real-time PCR.

Results Patients with diabetes complications had significantly shorter leukocyte telomere length than both patients without diabetes complications and healthy control subjects. Moreover, among patients with diabetes complications, leukocyte telomere length became significantly and gradually shorter with the increasing number of diabetes complications. The magnitude of the effect of the decrease of the abundance of telomeric template vs. a single-copy gene length (T/S ratio) on complications is described by the estimated odds ratio OR = 5.44 (95% CI 3.52–8.42).

Conclusions The results of the study support the hypothesis that telomere attrition may be a marker associated with the presence and the number of diabetic complications.

Abbreviation T/S, telomeric template vs. a single-copy gene

Introduction

Patients with Type 2 diabetes mellitus have a marked increase in risk of developing micro- and macrovascular complications, and many different factors have been associated with this increased risk [1]. Firstly, glycaemic control has a larger impact on the occurrence of endothelial damage and progression of complications [2]. Other metabolic disorders tend to coexist in these patients, such as obesity, hypertension and lipid abnormalities, which include reduction in HDL cholesterol [3]. However, neither a single diabetes-related risk factor or marker, nor a cluster of them seem to explain the increased risk for diabetic complications as a whole. On this basis, new markers would be very useful as a better predictor of the development of diabetic complications, especially if able to focus on multiple targets of disorders or damage as a result of diabetes.

In recent years, the role of telomere length in the pathogenesis of cardiovascular disease and diabetes has attracted a continuously growing research interest. Telomeres, the tandem repeats of the TTAGGG DNA sequence extending at the end of the eukaryotic chromosomes, undergo attrition during every cell division and their length is the best indicator of the replication potential of somatic cells [4]. Cells reaching a critical short telomere length or harbouring otherwise dysfunctional telomeres become senescent or apoptotic, while cells that must continue their division for many generations, such as stem cells, prevent this shortening by the activity of the ribonucleoprotein enzyme telomerase [4]. Senescence and stem cell ageing are influenced not only by telomere shortening but also by ongoing accumulation of DNA damage, which may be more prominent at the cytosine–phosphate–guanine (CpG) site of the telomere than other DNA sites [4]. Thus, replication senescence pathways exploit the same mechanisms that recognize DNA breaks and induce stalling of the cell cycle and, potentially, repair of the lesion [5]. Several lines of evidence support the hypothesis that gradual telomere attrition, which appears to be a normal part of ageing, is accelerated in cells that are exposed to internal or external stressors, known to provoke increased cellular proliferation and high oxidative stress [6]. Many investigations have used leukocytes, the most readily available proliferative cells in humans, in order to explore associations between telomere lengths and human diseases. Leukocyte telomere

length is highly variable among individuals, as a consequence of the high inter-individual variations at birth, as well as the replicative history of haematopoietic stem cells and progenitor cells after birth [7]. Haematopoietic stem cells are expected to be particularly vulnerable to telomere shortening and accelerated ageing because of their rapid proliferation and high oxidant species production during inflammatory response**.** Thus, the cumulative burden of inflammation and oxidative stress over the individual's lifespan reflects the telomere dynamics of haematopoietic stem cells and all their proxies, including peripheral leukocytes [7]. Many epidemiological and clinical studies have found that a large host of human age-related diseases and related risk factors are associated with leukocyte telomere length [8].

Interestingly, telomere length also seems like a useful marker for Type 2 diabetes. It has been reported that telomeres were shorter in patients with Type 2 diabetes compared with control subject [9–11], even if the shortening was attenuated in patients with well-controlled diabetes [12]. In addition, telomere shortening has been linked to some diabetes complications, such as diabetic nephropathy [13], microalbuminuria [14] and epithelial cancers [15]. Moreover, a very interesting finding, confirmed in independent studies, including our previous one, is that patients with diabetes and atherosclerotic manifestations have the shortest telomeres compared with patients with diabetes or cardiovascular diseases alone (16–18). Finally, a recent study suggests that telomere shortening is associated with a poor prognosis in patients with Type 1 diabetes [19].

Taking these considerations as a starting point, the aim of our study was to verify whether leukocyte telomere length is associated with the presence and the number of diabetic complications in a large sample of patients with Type 2 diabetes.

Patients and methods

Study sample

Five hundred and one patients with Type 2 diabetes [mean age (SD), 65.6 (8.2) years] and 400 healthy control subjects [mean age (SD), 65.1 (8.2) years] were enrolled after informed consent was obtained from each subject. The study protocol was approved by the local Review Board. Sites of recruitment

were in the central part of Italy, including the cities of Ancona, Ascoli Piceno, Macerata, Fermo and Pesaro-Urbino. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria [20]. Inclusion criteria for patients with diabetes were: BMI < 40 kg/m^2, age 35–85 years, ability and willingness to give written informed consent and to comply with the requirements of the study. Information collected included data on vital signs, anthropometric factors, medical history and behaviours as well as physical activity. DNA was collected from participants who provided consent for the use of genetic material (100% of the sample). The presence/absence of diabetic complications was evidenced as follows: diabetic retinopathy by fundoscopy through dilated pupils and/or fluorescence angiography; incipient nephropathy, defined as a urinary albumin excretion rate > 30 mg/24 h and a normal creatinine clearance; renal failure, defined as an estimated glomerular filtration rate < 60 ml/min per 1.73 m^2; neuropathy established by electromyography; ischaemic heart disease defined by clinical history, and/or ischaemic electrocardiographic alterations; peripheral vascular disease, including atherosclerosis obliterans and cerebrovascular disease on the basis of history, physical examinations and Doppler velocimetry.

Among the 284 patients with Type 2 diabetes with at least one complication, 102 were affected by neuropathy, 35 by peripheral vascular disease, 25 by cerebrovascular disease, 96 by cardiovascular ischemia, 71 by nephropathy, 150 by retinopathy and 21 by renal failure.

Healthy subjects have been selected from a larger population of subjects belonging to a diabetes prevention programme. Information on their healthy state was assessed by questionnaires, laboratory assays and physical examination [21].

Hypertension was defined as a systolic blood pressure > 140 mmHg and/or a diastolic blood pressure > 90 mmHg, measured while the subjects were sitting, and which was confirmed on at least three different occasions. All the selected subjects consumed a Mediterranean diet. Overnight fasting venous blood samples of all subjects were collected from 08.00 to 09.00 h. The samples were either analysed immediately or stored at –80 °C for no more than 30 days.

Laboratory assays

Blood concentrations of total and HDL cholesterol, triglycerides, fasting glucose, HbA_{1c}, fasting insulin, fibrinogen, high-sensitivity C-reactive protein (hsCRP), creatinine, urea nitrogen and white blood cells were measured by standard procedures. An immunoenzymatic method for the plasminogen activator inhibitor-1 (PAI-1) antigen was used (Biopool, XXXXX, XXXXX).

Measurement of telomere length

High molecular weight DNA was isolated from white blood cells using a Qiagen kit for DNA extraction (XXXXXX, XXXXXX, XXXX). Telomere length was measured as comparative quantification, in particular as abundance of telomeric template vs. a single-copy gene (T/S) by quantitative real-time PCR as described by Cawthon *et al*. [22] with some modifications. For each individual in whom the T/S was assayed, a 5-µl aliquot with 20 ng DNA and 10 µl of master mix were added. For each standard curve, one reference DNA sample was diluted serially in water by 1.68-fold per dilution to produce five concentrations of DNA ranging from 30 to 2 ng in 5 µl. The telomere and single-copy gene (36B4) were analysed on the same plate in order to reduce interassay variability.

PCR primers sequences and concentration for telomere and 36B4 were reported by Cawthon *et al*. [22].

The thermal cycling profile was: (1) one cycle of 10 s at 95 °C; (2) 30 cycles of 5 s at 95 °C, 15 s at 57 °C and 20 s at 72 °C.

Measurements were performed in duplicate and reported as T/S ratio relative to a calibrator sample (Roche, XXXXXX) to allow comparison across runs. All PCRs were performed on the real-time Chromo4 MJ Research system (Bio-Rad Laboratories, Hercules, CA, USA).

The coefficients of variations within duplicates of the telomere and single-gene assay were 2% and 1.8%, respectively. Approximately 30% of samples were repeated on different plates to assess the T/S reproducibility. The interassay coefficient of variation was $< 10\%$. All analysis was undertaken blinded.

The correlation coefficient between T/S and the telomere restriction fragment was $R^2 = 0.88$.

Statistical analysis

Data were analysed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Triglyceride, insulin, hsCRP and telomere length levels were log transformed before statistical analyses to achieve a normal distribution. Their results are shown as median and interquartile range. Differences among groups were compared by univariate analysis using one-way analysis of variance (ANOVA) for continuous variables and the χ^2-test for categorical variables. The Scheffè post hoc test was used for multiple comparisons. Pearson partial correlation coefficients were calculated to analyse the association between leukocyte telomere length and the other independent variables. The ANOVA trend analysis with polynomial contrast was applied to assess whether there was a functional relationship between the leukocyte telomere length and the number of diabetes complications. This analysis was adjusted for the covariates that showed a correlation coefficient greater or equal to 0.15 with the leukocyte telomere length. The ordinal logistic regression model was then fitted to find the relationship between the increase in the number of diabetes complications (ordinal outcome) and leukocyte telomere length. The ordinal outcome was subdivided into five categories: Type 2 diabetes without complications (T2DM–), presence of one, two, three and more than three diabetic complications (T2DM+1, T2DM+2, T2DM+3 and T2DM>3, respectively). The proportional odds model was used. The assumption made to conduct this analysis was that the regression lines for the different outcome categories were parallel to each other. This assumption was satisfied when the score test for the proportional odds (χ^2) was greater than 0.05.

Finally, the predicted probabilities of the number of diabetic complications vs. leukocyte telomere length, estimated from the proportional odds model, were plotted. In all statistical analyses, a probability value less than 0.05 was considered statistically significant.

Results

Table 1 shows the main characteristics of the three studied groups of subjects: control subjects, patients with Type 2 diabetes without complications (T2DM–) and patients with Type 2 diabetes with a range of 1–7 different complications (T2DM+).

We selected a control group with the same median age as the patients with Type 2 diabetes (Type 2 diabetes, mean age ± SD, 65.6 ± 8.2 years vs. control subjects, mean age ± SD, 65.1 ± 8.2 years, $P > 0.05$) and with the same percentage of men (Type 2 diabetes, $n = 299$, 59.7%, vs. male control subjects, $n = 220$, 55.6%, $P > 0.05$). However, as T/S was inversely correlated with age ($r = –0.15$; $P < 0.001$) and it was significantly higher in women than in men [median (interquartile range) 0.43 (0.32–0.59) and 0.42 (0.32–0.56), respectively; F-test = 12.1, d.f. = 1, $P = 0.001$), all the subsequent analysis were adjusted for age and sex.

No statistical significance was found between hypoglycaemic therapy and T/S in patients with Type 2 diabetes ($F = 1.04$, $P = 0.30$).

Analysis of variance among patients with Type 2 diabetes with and without complications and control subjects indicated that significantly shorter telomere lengths (adjusted for age and sex) were present in patients with Type 2 diabetes with complications (F-test = 40.6, d.f. = 2, $P < 0.001$) compared with control subjects and patients with diabetes without complications (Table 1). No significant difference was observed between control subjects and patients with Type 2 diabetes without complications in T/S (Table 1).

The T/S correlation with selected variables is reported in Table 2. Variables that showed a Pearson correlation coefficient with the T/S greater or equal to 0.15 were: glucose (Pearson correlation coefficient = –0.16, $P < 0.001$), HbA_{1c} (Pearson correlation coefficient = –0.19, $P < 0.001$) and creatinine (Pearson correlation coefficient = –0.18, $p < 0.001$). A weak correlation was observed between T/S and duration of diabetes (Pearson correlation coefficient = –0.14, $P = 0.003$). Consequently, the analysis of variance among patients with Type 2 diabetes with and without

complications and control subjects was adjusted for age, sex, glucose, HbA_{1c} and creatinine and confirmed the above-mentioned results (see also Supporting Information, Table S1).

We then evaluated the functional relationship between telomere length and the number of the evaluated diabetic complications. Patients with Type 2 diabetes with complications were arranged by the presence of one, two, three and more than three diabetic complications. The ANOVA, adjusted for sex and for the covariates that showed a correlation coefficient greater or equal to 0.15 with the T/S, such as age, glucose, HbA_{1c} and creatinine, clearly showed that T/S telomere length was significantly related to the number of complications (F-test = 11.9, d.f. = 5, $P < 0.001$).

The adjustment for the covariates that showed a significant correlation coefficient lower than 0.15 with the T/S, such as waist–hip ratio, total cholesterol, HDL cholesterol, triglycerides and duration of diabetes, did not changed these results (see also Supporting Information, Tables S2, S3 and S4).

The trend analysis evidenced a significant linear relationship between T/S telomere length and the numbers of diabetes complications (P for trend < 0.001) (Fig. 1). An ordinal logistic regression model was fitted to analyse the association between the increase in number of diabetes complications (ordinal outcome) and the decrease in leukocyte telomere length. The ordinal outcome was subdivided into five categories: T2DM–, T2DM+1, T2DM+2, T2DM+3 and T2DM>3. The score test for the proportional odds assumption yielded $P = 0.404$ ($\chi^2 = 2.918$, d.f. = 3), indicating that the model is appropriate for the data. The magnitude of the effect of the decrease of T/S length on complications is described by the estimated odds ratio OR = 5.44 (95% CI 3.52–8.42).

The probability of presenting complications in association with decreasing T/S lengths is shown in Fig. 2. The risk of developing any diabetes complications was higher in those patients with shorter telomere length compared with those with higher telomere length. Figure 2 depicts the predicted probabilities, yielding a greater descriptive insight about the effect of T/S lengths on diabetes complications. The probability of having diabetes complications increased with decreasing T/S lengths.

Discussion

The main finding of the present study is that leukocyte telomere length is associated with the presence and the number of diabetic complications in a large sample of Caucasian patients with Type 2 diabetes. These results are in accordance with the growing body of literature evidencing that premature cell senescence is an important cause and/or consequence of Type 2 diabetes and its complications [18, 23]. Diabetes mellitus is known to be able to trigger the pathways responsible for hyperglycaemia-induced cell damage that cause accelerated telomere loss during cell division [12]. Based on our findings, we can therefore hypothesize that shorter telomeres, leading to senescent phenotypes in multiple cell types, not only promote the onset of diabetes, but are also strictly linked to the presence of overt vascular damage [18]. Figure 2 shows a probabilistic model of developing diabetes complications with the decreasing leukocyte telomere length. This relationship between telomere length and spreading of damage provides an insight into the potential of using telomere length for the risk assessment of Type 2 diabetes complications.

Interestingly, no significant difference was observed in leukocyte telomere length between patients with Type 2 diabetes without complications and control subjects. These findings are in accordance with ones from previous studies showing reduced leukocyte telomere length length in patients with Type 2 diabetes with different complications but not in patients with Type 2 diabetes with a well-controlled glucose metabolism [12]. Moreover, our results add new data regarding the association between telomere length and progression of Type 2 diabetes, enforcing the suggestion that leukocyte telomere length could be an interesting marker associated with the presence and the number of diabetic complications.

Until now, the amount of patients with Type 2 diabetes and control subjects analysed in our study to identify the correlation between leukocyte telomere length and diabetes complications is the largest ever reported in the literature [13–16, 24].

The inverse correlation of leukocyte telomere length with variables reflecting the glycaemic state of patients observed in our previous study is confirmed by the present results, suggesting that

hyperglycaemia might be causing the telomere loss in diabetes and its complications [18]. In particular, the inverse correlation between leukocyte telomere length and HbA_{1c} and glucose clearly suggests this hypothesis. A plausible explanation is that the shorter leukocyte telomere length reflects an increased white blood cell turnover as a consequence of the chronic inflammation and oxidative stress that accompany diabetes and its complications. It was demonstrated that the quantification of telomere length from peripheral blood cells is an indicator of stem cell divisions. In fact, the age-dependent decline in telomere length of mononuclear bone marrow cells was found to be very similar compared with peripheral blood cells, suggesting an ageing process already in stem and progenitor cells [18]. The contribution of inflammation to the ageing process of peripheral and bone marrow stem cells cannot be excluded, although many previous studies, including Salpea and co-workers and ours, have failed to detect an association between leukocyte telomere length and some important inflammatory markers [10,16,18]. No significant inverse correlation between leukocyte telomere length and inflammatory markers, including hsCRP, fibrinogen and PAI-1, were observed in our study. However, such findings may be explained by the fact that approximately 40% of the patients with Type 2 diabetes with complications were using anti-inflammatory drugs, which have well-documented actions on the above-mentioned measurements, suggesting that the correlation between telomere length and inflammatory markers probably was blunted by the anti-inflammatory therapy received by the patients with Type 2 diabetes, especially in those with complications.

There is a need for prospective studies to establish whether telomere shortening is a cause or a consequence of diabetes and examine the usefulness of leukocyte telomere length in predicting disease risk, especially for diabetes, as there is no previous record. Recently, it was reported that individuals in families with exceptional longevity have better maintenance of telomere length and that the telomerase genes may function as important genetic determinants of both human longevity and telomere length [25]. These observations provide an insight into the theory that predisposition to cardiovascular disease and/or diabetes might be expressed through inherited short telomeres and/or through inherited genetic variants of the telomerase gene with different activity in the maintenance of leukocyte telomere length.

In fact, in a recent longitudinal study of patients with stable coronary artery disease, not only were telomere shortening and maintaining observed but also telomere lengthening, suggesting an involvement of telomerase activity in leukocyte telomere length trajectories [25]. However, if the leukocyte telomere length potential for the risk assessment of Type 2 diabetes complications development will be confirmed, the leukocyte telomere length determination could be included in the pattern of variables useful for the clinical management of patients with Type 2 diabetes.

Limitations of the study

Limitations of our study need to be considered. The possibility that an unmeasured factor, such as other inflammation markers or lifestyle factors, might confound the observed effects of Type 2 diabetes complications on telomere length cannot be excluded. Moreover, this study does not include any data on telomere loss over time, as all measurements were taken at a certain time point and therefore the rate of telomere attrition in patients with diabetes could not be addressed. Finally, no definite conclusions can be made on whether the observed shorter telomeres in patients are a cause or a consequence of diabetes complication as this was a cross-sectional study. Moreover, our study does not explain the mechanisms responsible for the short leukocyte telomere length in the diabetic complications.

Competing interests

Nothing to declare.

Acknowledgements

This work was supported in part by a grant from Italian Health Ministry fund, FIRB project code RBNE018AAP_007. We thank Michela Cucchi, Roberta Bacchiocchi and Valeria Stecconi for the technical support.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. T/S mean (SD) adjusted by age, sex, glucose, HbA_{1c} and creatinine in control subjects and patients with Type 2 diabetes with and without complications.

Table S2. T/S mean (SD) adjusted by waist–hip ratio, total cholesterol, HDL cholesterol, duration of diabetes and triglycerides in control subjects, patients with Type 2 diabetes without complications and those with one, two, three and more than three complications.

Table S3. T/S mean (SD) in patients with Type 2 diabetes with and without complications adjusted by duration of diabetes.

Table S4. Mean T/S (SD) in patients with Type 2 diabetes without complications and those with one, two, three and more than three diabetic complications, adjusted for duration of diabetes.

Figure 1 Box plot of leukocyte telomere length (T/S) according to the number of diabetic complications. The boxes represent the median (the band) and the interquartile range with the vertical lines showing the minimum and maximum data values: control subjects (CTR); patients with Type 2 diabetes without complications (T2DM–); patients with Type 2 diabetes in the presence of one, two, three and more than three diabetic complications (T2DM+1, T2DM+2, T2DM+3 and T2DM>3, respectively).

Figure 2 Plot of probabilities of diabetic complications number vs. leukocyte telomere length (T/S) estimated from the proportional odds model. The probability of having diabetic complications increases with the decreasing of leukocyte telomere length (T/S).

Table 1 Biochemical and clinical variables of healthy control subjects (CTR, $n = 400$) and patients with Type 2 diabetes without (T2DM–, $n = 217$) and with (T2DM+, $n = 284$) complications; total $n = 901$

Variables	~~CTR~~Control	~~T2DM–~~Type 2 diabetes without complications	~~T2DM+~~Type 2 diabetes with complications	*P*-value*
Age, years	65.1 (8.2)	65.9 (8.3)	66.9 (7.8)	0.38
BMI, kg/m^2	26.9 (4.7)‡	29.3 (4.9)	28.7 (4.4)	< 0.001
Waist–hip ratio	0.88 (0.08)‡	0.94 (0.07)	0.94 (0.07)	< 0.001
Total cholesterol, mmol/l	5.6 (0.9)	5.4 (0.8)	5.2 (0.9)†	< 0.001
HDL cholesterol, mmol/l	1.8 (0.4)‡	1.4 (0.3)	1.3 (0.4)	< 0.001
Triglycerides, mmol/l	0.95 (0.68–1.42)‡	1.33 (0.90–1.71)	1.25 (0.95–1.88)	< 0.001
Glucose, mmol/l	5.2 (0.5)§	8.6 (2.3)§	9.5 (2.9)§	< 0.001
HbA_{1c}, mmol/mol	39 (4)	55 (4)	61 (5)	< 0.001
HbA_{1c}, %	5.7 (0.4)§	7.2 (1.2)§	7.7 (1.3)§	
Insulin, pmol/l	35.6 (23.0–48.3)	41.5 (26.0–64.1)¶	35.3 (22.6–54.0)	< 0.001
White blood cells, 10^9/l	6.4 (1.5)‡	6.6 (1.5)	6.8 (1.7)	< 0.001
PAI-1, ng/ml	22.0 (10.9)	21.6 (10.3)	19.6 (8.9)†	0.04
hsCRP, mg/l	1.7 (0.8–3.7)‡	2.6 (1.2–5.9)	2.4 (1.2–4.6)	< 0.001
Fibrinogen, g/l	2.8 (0.7)	3.2 (0.8)	3.0 (0.7)	0.19
Creatinine, µmol/l	72.5 (27.4)	75.4 (16.8)	88.4 (34.5)†	< 0.001
Urea nitrogen, mmol/l	105.5 (24.9)	107.5 (26.8)	120.1 (43.6)†	< 0.001
Telomere length, T/S	0.45 (0.34–0.60)	0.46 (0.36–0.61)	0.36 (0.27–0.49)†	< 0.001
Duration of diabetes, years		13.5 (9.5)	20.4 (11.5)	< 0.001
Males, % (*n*)	55.0 (220)	55.8 (121)	62.7 (178)	0.11
HbA_{1c} > 53 mmol/mol (> 7%), % (*n*)		51.9 (112)	66.5 (189)	< 0.001
Current smokers, % (*n*)	16.2 (65)	13.9 (30)	15.2 (43)	0.43
Use of statins, % (*n*)	6.5 (26)	15.6 (34)	22.5 (64)	< 0.001
Use of sulphonylurea, % (*n*)		38.0 (82)	56.7 (161)	< 0.001
Use of metformin, % (*n*)		33.8 (73)	40.5 (115)	< 0.001
Use of insulin, % (*n*)		7.6 (16)	27.1 (77)	< 0.001
Use of anti-inflammatory, % (*n*)	6.2 (25)	11.8 (25)	38.4 (109)	< 0.001
Hypertension, % (*n*)	31.5 (126)	56.5 (122)	71.1 (202)	< 0.001

Variables are expressed as mean (SD) for normally distributed variables, as median (IQR: 25th–75th percentiles) for log transformed variables and as percentage (*n*) for categorical variables.

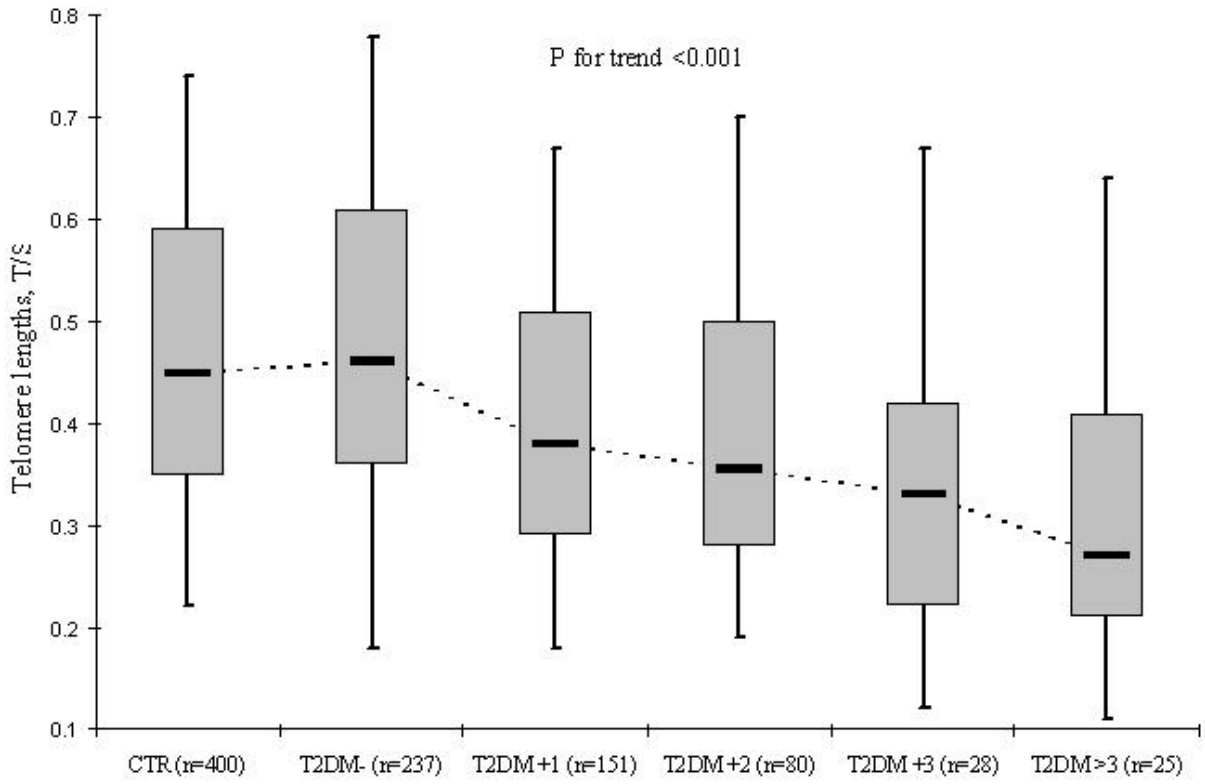
**P* from ANOVA for continuous variables and from χ^2 -test for dichotomic variables.

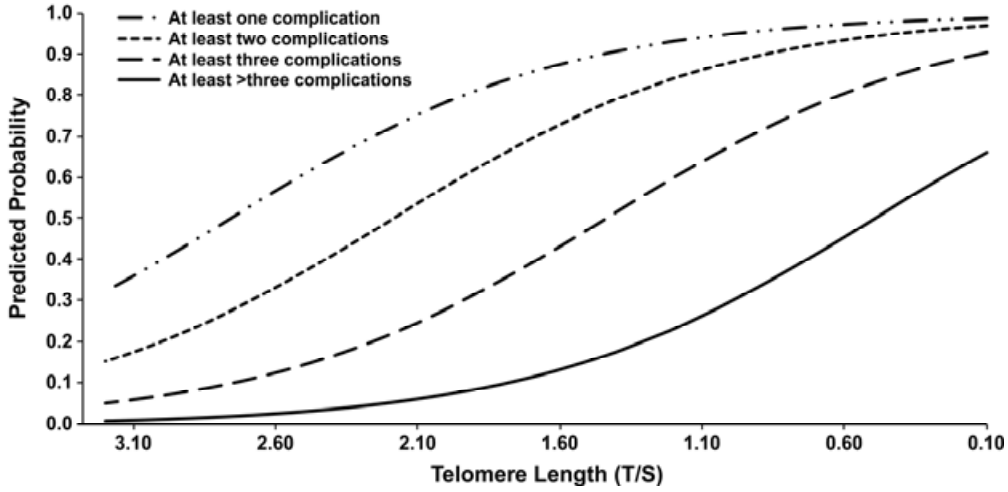
† T2DM+ vs. T2DM– and CTR, $P < 0.05$.
‡ CTR vs. T2DM– and T2DM+, $P < 0.05$.
§ CTR vs. T2DM– vs. T2DM+, $P < 0.05$.
¶ T2DM– vs. T2DM+ and CTR, $P < 0.05$.
hsCRP, high-sensitivity C-reactive protein; PAI-1, plasminogen activator inhibitor-**1**; T/S, **telomeric template vs. a single-copy gene**.

Table 2 Pearson correlation coefficients between T/S and selected variables

Variables	T/S	*P*-value
Age	–0.15	< 0.001
BMI	–0.02	0.598
Waist–hip ratio	–0.11	0.011
Total cholesterol	0.09	0.075
HDL cholesterol	0.10	0.041
Triglycerides	–0.10	0.041
Glucose	–0.16	< 0.001
HbA_{1c}	–0.19	< 0.001
Insulin	0.04	0.371
White blood cells	–0.08	0.091
PAI-1	0.05	0.261
hsCRP	–0.03	0.511
Fibrinogen	–0.04	0.376
Creatinine	–0.18	< 0.001
Mean urea nitrogen	–0.08	0.091
Duration of diabetes (only patients with diabetes)	–0.14	0.003

hsCRP, high-sensitivity C-reactive protein; PAI-1, plasminogen activator inhibitor-1; T/S. telomeric template vs. a single-copy gene.


P for trend <0.001
Telomere lengths, T/S
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
CTR (n=400)
T2DM- (n=237)
T2DM+1 (n=151)
T2DM+2 (n=80)
T2DM+3 (n=28)
T2DM>3 (n=25)


At least one complication
At least two complications
At least three complications
At least >three complications
Predicted Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3.10
2.60
2.10
1.60
1.10
0.60
0.10
Telomere Length (T/S)