

Telomere Dysfunction–Related Serological Markers Are Associated With Type 2 Diabetes

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OBJECTIVE—Recent studies have identified a set of serological markers for telomere dysfunction and DNA damage. The relevance of these serological markers in type 2 diabetes remains elusive. We investigated the association of serological markers (elongation factor 1 α [EF-1 α], stathmin, and *N*-acetyl-glucosaminidase) with leukocyte telomere length, a functional variant of uncoupling protein-2 (*UCP2*), and susceptibility of type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 930 patients and 867 control subjects were recruited to examine the association between leukocyte telomere length, *UCP2* variant (–886G>A), recently identified serological markers, and type 2 diabetes. Telomere length was determined by a quantitative real-time PCR–based assay. EF-1 α , stathmin, and C-reactive proteins were measured by enzyme-linked immunosorbent assays. *N*-acetyl-glucosaminidase was measured by an enzyme activity assay. The *UCP2* variant was determined by PCR and restriction enzyme digestion.

RESULTS—The average telomere length of type 2 diabetic patients was significantly shorter than that of control subjects. Serological *N*-acetyl-glucosaminidase correlates with both age and telomere length and was significantly higher in patients than in control subjects. Neither EF-1 α nor stathmin showed significant difference between patients and control subjects. The *UCP2*–886G>A variant correlated with type 2 diabetes status but did not correlate with telomere length or the serological markers. Multivariate analysis showed that higher serological *N*-acetyl-glucosaminidase, shorter telomeres, and the *UCP2*–886G>A variant are independent risk factors for type 2 diabetes.

CONCLUSIONS—Serological *N*-acetyl-glucosaminidase, telomere length, and the *UCP2*–886G>A variant are independent risk factors for type 2 diabetes. Serological *N*-acetyl-glucosaminidase correlates with telomere length but not with the *UCP2*–886G>A variant.

Diabetes Care 34:2273–2278, 2011

Type 2 diabetes is the most common form of diabetes, characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. In China, type 2 diabetes has become a major public health problem because of the rapid change of lifestyle in the Chinese population (1). Epidemiology

studies have identified a number of classic risk factors for type 2 diabetes, including age, obesity, and unhealthy lifestyles. Recently, several cross-sectional studies suggested that shorter telomeres correlate not only with the risk of type 2 diabetes but also with its complications, such as microalbuminuria and myocardial infarction

(2–4). Experimental evidence showed that DNA damage responses and cellular senescence induced by telomere dysfunction led to increased reactive oxygen species (ROS) in fat cells, which ultimately resulted in the activation of p53, inflammation responses, and the promotion of insulin resistance (5). Therefore, genomic damage, accumulation of ROS, and shorter telomeres may be linked to the onset and progression of diabetes and its complications. A proper evaluation of the cumulative burden of internal and external stress from oxidative damage and telomere attrition could be very useful in clinical settings.

The average telomere length of peripheral leukocytes reflects not only the birth telomere length but also the accumulation of inflammation (6) and oxidative stresses of the body (7,8). Thus, shorter telomeres could be considered as a useful marker for assessing the accumulative risk for aging-associated human diseases. We have recently identified a set of biomarkers (cathelin-related antimicrobial peptide [CRAMP], stathmin, elongation factor 1 α [EF-1 α], and chitinase) that are induced by telomere dysfunction or γ -irradiation (9). Although the functional role of these biomarkers in response to DNA damage or telomere dysfunction remains to be investigated, these proteins could represent useful biomarkers in aging and associated diseases because the expression of these proteins can easily be measured in human blood serum using enzyme-linked immunosorbent assays and enzyme activity assays.

Leukocyte telomere length is influenced by both genetic and environmental factors. Differences in leukocyte telomere length have been observed in different ethnic populations (10). Most of the epidemiological studies about telomere length and type 2 diabetes were conducted in white populations. A study with a relatively small sample size was conducted in the Asian-Indian population (3). Thus far, the association of shorter telomeres and type 2 diabetes has not been identified in the Chinese population.

Uncoupling protein-2 (*UCP2*) is a widely expressed inner mitochondrial membrane protein. Multiple variants of

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Received 25 December 2010 and accepted 18 July 2011.

DOI: 10.2337/dc10-2431

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the *UCP2* gene have been implicated in obesity and diabetes (11,12). It was reported that the common $-886G>A$ variant in the promoter of the human *UCP2* gene, which enhances its transcriptional activity, is associated with reduced ATP production and insulin secretion. A recent study showed that those carrying the functional promoter variant $-866A$ of *UCP2* have shorter telomeres (8). We also investigated whether the *UCP2*– $886G>A$ variant is associated with telomere length and type 2 diabetes in a Chinese population.

In this study, we investigated the association of leukocyte telomere length, the biomarkers of telomere dysfunction and DNA damage (stathmin, EF-1 α , and *N*-acetyl-glucosaminidase), the oxidative stress–related functional variant (*UCP2*– $886G>A$), C-reactive protein (CRP), and lipid profile in type 2 diabetic (T2D) patients in a Chinese population. Our data indicated that shorter telomeres and serological *N*-acetyl-glucosaminidase levels are independent risk factors for type 2 diabetes. The *UCP2*– $886G>A$ variant correlated with type 2 diabetes but not with age-adjusted leukocyte telomere length or serological *N*-acetyl-glucosaminidase levels. These findings suggest that serological *N*-acetyl-glucosaminidase is a potential indicator for shorter telomeres and type 2 diabetes.

RESEARCH DESIGN AND METHODS

T2D patients and 867 healthy control subjects were recruited from urban community centers in China. Informed consent was obtained from all subjects at the start of the study. Patients' data were evaluated anonymously. The study was designed in accordance with the principles of the Declaration of Helsinki and was approved by an institutional review committee. All participants underwent complete physical examinations, as well as hematological, biochemical, and instrumental examinations at the time of blood collection. Venous blood was obtained from the antecubital vein from all participants after a 10–12-h overnight fast for the measurement of plasma glucose, lipid panel, and DNA extraction. A detailed medical history of each subject was obtained, including past and present medical history, list of current medications, and smoking habits. Subjects were divided into three groups (nonsmokers, ex-smokers, and current smokers). Smoking was used as a quantitative variable (number of packs smoked per month) in

multivariable analysis. T2D patients were diagnosed according to World Health Organization criteria (13). Patients with diabetic retinopathy or diabetic nephropathy were excluded from the study. All participants were free of infectious diseases and malignancy.

Biochemical assays

Blood specimens were centrifuged immediately; serum and plasma samples were stored at -80°C without repeat freeze-thaw cycles until they were analyzed within 6 months. High-sensitivity CRP levels were assayed by the immunoturbidometric method (DiaSys Diagnostics, Holzheim, Germany) on the Hitachi 7600-010 Automatic Clinical Analyzer. The assay could detect a minimal CRP concentration of 0.05 mg/L, and values below this level were classified as undetectable. Blood concentrations of total, HDL, and LDL cholesterol; triglycerides (TGs); and fasting glucose were measured by routine laboratory methods. Serological levels of stathmin and EF-1 α were determined by enzyme-linked immunosorbent assays. Serological levels of *N*-acetyl-glucosaminidase were measured by the chitinase activity assay kit, as described previously (9,14).

Measurement of telomere length

The integrity of the DNA sample was assessed by the electrophoresis of 0.5 μg of undigested DNA on 1.0% agarose gel. The telomere length ratio (T/S ratio) was measured using a quantitative PCR method, as described previously (15). Individual DNA samples were measured in triplicate. A reference DNA sample (pool of genomic DNA from 100 subjects) was included in each measurement to control interassay variability. The coefficients of variation for the triplicate measurement were 3.6% for telomere and 2.8% for the single-gene assay. Test samples with threshold cycle numbers that fell outside the range defined by the standard curves were rerun at different concentrations to ensure that they were amplified within the linear range. All analyses were conducted blind to the disease status of the individuals.

The relative telomere length was calculated with slight modifications, according to the previously published method (15). The linear regression line between mean telomere length (measured by terminal restriction fragment analysis based on Southern blotting) and the T/S ratio (measured by the quantitative PCR–based

method) was obtained, as described previously (14,16), and used for the calculation of the corresponding telomere length in base pairs (bp) from the T/S ratio measured in each subject.

UCP2 single nucleotide polymorphism genotyping

The $-866G>A$ promoter variant of the *UCP2* gene was genotyped using a PCR-based method, followed by restriction enzyme digestion and electrophoresis. A 278-bp fragment was amplified by forward primer 5'-GAGCGGCCTGGCGTTTAGGG-3' and reverse primer 5'-GT-TTGTCCCGGCCAGAGGGC-3', followed by *MluI* restriction enzyme digestion for 2 h. The genotypes were determined by electrophoresis on a 2% agarose gel as follows: GG genotypes were dissected by *MluI* and showed two bands of 237 and 41 bp; AA genotypes were not dissected and showed only one band of 278 bp; and GA genotypes showed three bands of 278, 237, and 41 bp. All samples were analyzed in duplicate.

Calculation and statistical analysis

Data were analyzed using the SAS program (version 9.1). Statistical significance was taken as $P < 0.05$, and all tests were two-tailed. Telomere length was log-transformed because of its skewed distribution. The χ^2 test was used for categorical variables, and one-way ANOVA was used for quantitative variables. Partial correlation coefficients and ANCOVA were used to adjust the comparison between groups. Furthermore, the multiple regression was used to derive unbiased estimates by controlling confounding factors. Logistic regression models were performed to calculate odds ratios (ORs) and 95% CIs on the basis of unadjusted assessment and then adjusted for age, sex, and other conventional risk factors.

RESULTS

Characteristics of study samples

Characteristics of T2D patients and control subjects at the time of blood donation are shown in Table 1. T2D patients and control subjects were matched by age and sex. BMI was significantly greater in T2D patients ($24.2 \pm 3.2 \text{ kg/m}^2$) compared with control subjects (23.1 ± 3.1 ; $P < 0.001$). T2D patients had significantly higher fasting plasma glucose and TGs and low HDL cholesterol compared with control subjects. Of interest, the total cholesterol (TC) level was not different

Table 1—Characteristics of T2D patients and control subjects

	Case subjects	Control subjects	P
N	930	867	
Age (years)	64.3 ± 8.6	64.1 ± 8.9	0.804
Male subjects (%)	29.9	30.5	0.797
Age of type 2 diabetes onset	57.0 (51.4–64.3)	—	
Duration of type 2 diabetes	5 (2.3–9)	—	
Current smokers (%)	23.4	21.0	0.276
BMI (kg/m ²)	24.2 ± 3.2	23.1 ± 3.1	<0.001*
Glucose (mmol/L)	8.2 ± 3.6	4.8 ± 1.0	<0.001*
TGs (mmol/L)	1.9 ± 1.5	1.5 ± 1.4	<0.001*
TC (mmol/L)	4.64 ± 1.03	4.69 ± 1.00	0.222
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.3 ± 0.3	<0.001*
LDL cholesterol (mmol/L)	3.0 ± 0.9	2.9 ± 0.9	0.282
CRP (mg/L)	2.5 ± 5.4	2.4 ± 6.9	0.671
UA (mmol/L)	280.7 ± 107.7	279.0 ± 92.3	0.731
Urea (mmol/L)	6.4 ± 2.0	5.8 ± 1.5	<0.001*
Creatinine (mmol/L)	84.3 ± 30.6	81.3 ± 15.7	0.010*
Hypertension (%)	54.6		
SBP (mmHg)	133.7 ± 17.5	124.6 ± 15.1	<0.001*
DBP (mmHg)	78.7 ± 9.8	76.4 ± 8.7	<0.001*
Family history (%)	12.5	2.3	<0.001*
Hypoglycemia medication (%)			
None	39.8	—	
Insulin	10.9	—	
Oral	53.6	—	
Both	4.2	—	
Telomere length (kb)	8.01 ± 1.83	8.59 ± 2.11	<0.001*

Data are means ± SD or median (interquartile range), where appropriate. * $P < 0.05$.

between the two groups. It is not surprising that urea and creatinine levels were higher in T2D patients compared with control subjects, perhaps because of advanced disease. Family history of diabetes was significantly higher in T2D patients compared with control subjects ($P < 0.001$).

Telomere length correlates with age and type 2 diabetes

The telomere length was calculated as described previously (14,16) and log-transformed to achieve a normal distribution before the following statistical analysis. Leukocyte telomere length was inversely correlated with age overall ($r = -0.09$; $P < 0.001$) as well as in the case group ($r = -0.073$; $P = 0.023$) and in the control group ($r = -0.115$; $P = 0.001$). Telomere length was shorter in male subjects compared with female subjects in the control group (8.37 vs. 8.69 kilobases [kb]; $P = 0.036$), whereas in T2D patients such a difference was not significant ($P = 0.193$). In the following analysis, telomere length was adjusted for age and sex. Significant differences in age- and sex-adjusted telomere length were observed between

T2D patients (8.012 kb [95% CI 7.901–8.141]) and control subjects (8.587 [8.444–8.730]; $P < 0.001$).

Serological N-acetyl-glucosaminidase correlates with age and type 2 diabetes

The serological levels of DNA damage and telomere dysfunction markers (EF-1 α , stathmin, and N-acetyl-glucosaminidase) were log-transformed to achieve a normal distribution before the following statistical analysis. Overall, only N-acetyl-glucosaminidase was positively correlated with age ($r = 0.150$; $P < 0.001$). N-acetyl-glucosaminidase was positively correlated with age in the case group ($r = 0.085$; $P = 0.010$) and in the control group ($r = 0.226$; $P < 0.001$). Stathmin was only positively correlated with age in the control group ($r = 0.114$; $P = 0.001$) but not in the case group. EF-1 α did not show correlation with age in any of the groups. Therefore, both stathmin and N-acetyl-glucosaminidase were age- and sex-adjusted in the subsequent analyses. Significant differences in serological N-acetyl-glucosaminidase were observed in T2D patients (92.846 ng/ μ L [95% CI 90.849–94.844]) compared with

control subjects (79.923 [78.254–81.592]; $P < 0.001$). The serological levels of EF-1 α or stathmin were not significantly different between case and control groups.

Correlation of telomere length and serological N-acetyl-glucosaminidase with conventional risk factors of type 2 diabetes

We next analyzed confounding factors that may affect the leukocyte telomere length and serological level of N-acetyl-glucosaminidase. The correlation between age-adjusted telomere length and other conventional risk factors are shown in Table 2. In total samples, sex, smoking (number of packs smoked per month), BMI, glucose, uric acid (UA), TC, stathmin, EF-1 α , and N-acetyl-glucosaminidase were significantly correlated with age-adjusted telomere length. Therefore, ANCOVA was used to adjust the comparison among groups, taking into account the effects of sex, smoking, BMI, UA, TC, stathmin, EF-1 α , and N-acetyl-glucosaminidase as covariates. After adjustment, the difference in age-adjusted telomere length between T2D patients and control subjects remained highly significant ($P < 0.001$). The correlation between age-adjusted N-acetyl-glucosaminidase and other conventional risk factors are shown in Table 3. In total samples, sex, smoking, exercise, systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose, urea, creatinine, TGs, HDL cholesterol, CRP, stathmin, and telomere length were significantly correlated with age-adjusted N-acetyl-glucosaminidase. Thus, ANCOVA was used to adjust the comparison among groups, taking into account the effects of sex, smoking, exercise, SBP, DBP, urea, creatinine, TGs, HDL cholesterol, CRP, stathmin, and telomere length as covariates. After adjustment, difference in age-adjusted N-acetyl-glucosaminidase between T2D patients and control subjects remained highly significant ($P < 0.001$).

Telomere length and serological N-acetyl-glucosaminidase are independent risk factors of type 2 diabetes

Logistic regression was performed to verify whether leukocyte telomere length and serological N-acetyl-glucosaminidase were independent risk factors of type 2 diabetes. Age-adjusted telomere length and age-adjusted N-acetyl-glucosaminidase were categorized according to the 50th

Table 2—Correlation coefficients of age-adjusted telomere length with classical risk factors in T2D patients and control subjects

	Total		Case subjects		Control subjects	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Sex	0.056	0.017*	0.043	0.192	0.072	0.035*
Smoking	−0.092	0.001*	−0.045	0.250	−0.132	0.001*
Exercise	0.027	0.332	0.026	0.505	0.032	0.420
BMI	−0.059	0.036*	0.008	0.846	−0.072	0.072
SBP	0.017	0.553	0.034	0.383	0.095	0.017*
DBP	<0.001	0.991	0.048	0.222	−0.001	0.987
Glucose	−0.098	<0.001*	0.005	0.896	−0.111	0.005*
Urea	−0.004	0.897	0.047	0.230	−0.014	0.718
Creatinine	−0.023	0.412	−0.031	0.431	<0.001	0.999
UA	−0.097	<0.001*	−0.105	0.007*	−0.098	0.014*
TC	0.081	0.004*	0.037	0.341	0.103	0.010*
TGs	−0.006	0.841	0.014	0.719	0.013	0.741
HDL cholesterol	0.037	0.183	−0.048	0.219	0.063	0.114
LDL cholesterol	−0.011	0.694	−0.015	0.700	−0.008	0.841
CRP	0.027	0.327	−0.017	0.667	0.060	0.133
Stathmin	−0.060	0.032*	−0.035	0.379	−0.077	0.054
EF-1α	−0.096	0.001*	−0.044	0.265	−0.146	<0.001*
N-acetyl-glucosaminidase	−0.066	0.019*	0.014	0.717	−0.087	0.029*

Stathmin, EF-1α, and N-acetyl-glucosaminidase are serological markers for telomere dysfunction and DNA damage. *P < 0.05.

percentile. The unadjusted OR of type 2 diabetes was 1.597 (95% CI 1.325–1.924) for individuals in the 50th percentile of shorter telomeres compared with those with longer telomeres (P < 0.001). In the stepwise logistic regression model, after adjusting for sex, smoking, BMI, UA, TC, stathmin, EF-1α, and N-acetyl-glucosaminidase, short telomere length

was confirmed as an independent risk factor for type 2 diabetes (1.775 [1.401–2.249]; P < 0.001). The unadjusted OR of type 2 diabetes was 2.132 (1.760–2.584) for individuals in the 50th percentile of high N-acetyl-glucosaminidase compared with those with low N-acetyl-glucosaminidase (P < 0.001). In the stepwise logistic regression model, after

adjustment of sex, smoking, exercise, SBP, DBP, urea, creatinine, TGs, HDL cholesterol, CRP, stathmin, and telomere length, high N-acetyl-glucosaminidase was confirmed as an independent risk factor for type 2 diabetes (2.022 [1.589–2.575]; P < 0.001). We did not observe any significant interactions in the aforementioned factors.

Table 3—Correlation coefficients of age-adjusted N-acetyl-glucosaminidase with classical risk factors in T2D patients and control subjects

	Total		Case subjects		Control subjects	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Sex	−0.063	0.009*	−0.075	0.025*	−0.058	0.096
Smoking	0.063	0.023*	0.067	0.088	0.051	0.200
Exercise	−0.074	0.008*	−0.093	0.017*	−0.066	0.096
BMI	−0.023	0.413	−0.110	0.005*	0.001	0.984
SBP	0.084	0.003*	0.047	0.231	0.003	0.941
DBP	0.086	0.002*	0.025	0.517	0.096	0.017*
Glucose	0.275	<0.001*	0.235	<0.001*	0.164	<0.001*
Urea	0.100	<0.001*	0.064	0.103	0.089	0.026*
Creatinine	0.089	0.001*	0.095	0.015*	0.073	0.067
UA	0.038	0.175	0.049	0.210	0.032	0.431
TC	−0.022	0.434	0.008	0.847	−0.027	0.504
TGs	−0.068	0.014*	−0.119	0.002*	−0.073	0.068
HDL cholesterol	0.057	0.040*	0.142	<0.001*	0.045	0.263
LDL cholesterol	0.045	0.111	0.046	0.239	0.046	0.252
CRP	0.062	0.025*	0.097	0.013*	0.033	0.404
Stathmin	0.166	<0.001*	0.139	<0.001*	0.196	<0.001*
EF-1α	0.043	0.128	−0.024	0.542	0.121	0.002*
Terminal restriction fragment	−0.066	0.019*	0.014	0.717	−0.087	0.029*

Stathmin, EF-1α, and N-acetyl-glucosaminidase are serological markers for telomere dysfunction and DNA damage. *P < 0.05.

UCP2-866G>A variants correlate with type 2 diabetes but not with telomere length or serological N-acetyl-glucosaminidase

The genotype distribution for the UCP2-866G>A variants was as expected from Hardy-Weinberg equilibrium ($P = 0.743$ for case subjects and $P = 0.066$ for control subjects). The distribution of the UCP2-866G>A variant between male and female subjects was not different ($P = 0.253$). The relationship between the -866G>A variant and other conventional risk factors was analyzed. There were no significant correlations between genotype distribution for the -866G>A variant and other conventional risk factors, with the exception of glucose ($P = 0.001$). The -866G>A variants were associated with type 2 diabetes (Cochran-Armitage trend test; $P = 0.017$). The frequency of the UCP2 -866A allele was 0.47 in case subjects and 0.51 in control subjects. The OR of the -866A allele compared with the -866G allele for type 2 diabetes was 1.172 (95% CI 1.029–1.335; $P = 0.016$). Carriers of the -866A allele have a 1.306-fold relative risk (OR) of type 2 diabetes compared with subjects with the GG genotype (1.058–1.612; $P = 0.012$). The difference of age-adjusted telomere length was not significant among different genotypes ($P = 0.735$ for case subjects and $P = 0.303$ for control subjects). Likewise, the age-adjusted serological N-acetyl-glucosaminidase did not show significant a difference between the carriers of the -866A allele and subjects with the GG genotype ($P = 0.087$ for case subjects and $P = 0.142$ for control subjects).

CONCLUSIONS—We investigated the association between leukocyte telomere length, the UCP2 variant (-866G>A), recently identified serological markers, and type 2 diabetes in a Chinese population. We found an age-dependent increase of serological N-acetyl-glucosaminidase and stathmin (biomarkers for DNA damage and telomere dysfunction) in control subjects, which is consistent with our previous work in whites (9,14). In contrast, we did not observe any significant correlation between serological EF-1 α and age. Such discrepancy may be attributed to differences in ethnicity and age. The previous study analyzed a cohort of 136 healthy whites aged 18–80 years (14) compared with 867 healthy Chinese subjects aged 45–89 years who were recruited from community-based populations.

In addition to their correlation with human aging, these serological markers have been implicated in age-related chronic diseases, such as liver cirrhosis and myelodysplastic syndrome (9). In the current study, we investigated the correlation of these serological markers with type 2 diabetes in a relatively large subject group. Compared with healthy control subjects, T2D patients showed significantly higher serological levels of N-acetyl-glucosaminidase but not stathmin or EF-1 α . After adjustment for age, sex, and conventional risk factors, a higher level of serological N-acetyl-glucosaminidase seems to be an independent risk factor for type 2 diabetes. The biological relevance of increased serological N-acetyl-glucosaminidase to the etiology of type 2 diabetes still is obscure. Increased activity of N-acetyl-glucosaminidase has been related to inflammation (17) and diabetes (18). Research done in mouse models suggested that insulin resistance and the resultant type 2 diabetes can be prevented by disabling the macrophage inflammatory pathway (19,20). In humans, insulin resistance is closely related to the presence of inflammatory cells in fatty tissue, where they produce cytokines (21). These cytokines cause the neighboring liver, muscle, or fat cells to become insulin resistant, which in turn can lead to type 2 diabetes. Recent experimental evidence suggested that telomere attrition provokes senescence-associated secretion of inflammatory cytokines (22). Experimental evidence from mouse models demonstrated that shorter telomeres induce the overproduction of cytokines leading to tissue stem-cell aging (23) and abnormal hematopoiesis (impaired lymphopoiesis and accelerated myelopoiesis) in telomerase knockout mice (24). The accelerated myelopoiesis induced by telomere dysfunction and aging could, in turn, enhance the activation of the macrophage inflammatory pathway in type 2 diabetes.

The UCP2 gene has been linked to mitochondrial ROS overproduction and diabetes (12). We examined the association of a functional UCP2 variant with telomere length and type 2 diabetes. In the current study, the frequency of the minor allele is much higher than in the previous study involving whites (37.2%) (11) but is similar to what has been observed in the Japanese population (48.9%) (25). In agreement with previous study (8), our data prove that both telomere length and the UCP2-866G>A variant are significantly associated with type

2 diabetes. Furthermore, we did not observe any significant correlation between telomere length and the UCP2-866G>A variant. This discrepancy may well be attributed to the ethnic difference in allele frequency.

It is worth mentioning the limitations of this study. First, there are multiple variants for UCP2, and we only tested the -866G>A variant. Therefore, we cannot exclude the possibility that other variants of UCP2 are associated with telomere length. Second, we did not measure the ROS, which is key in evaluating cross-talk between telomere dysfunction and oxidative stress. Third, population differences may be quite large between different ethnic groups (10), rendering it difficult to extrapolate to other Chinese populations, with the exception of the Han population. Last, the cross-sectional design of our study does not provide evidence for a causal relationship between T2D patients and shorter telomeres. A definitive answer to the question of whether telomere attrition is a primary cause or a consequence of type 2 diabetes requires prospective epidemiological studies. Considering the relevance of the current findings, telomere-associated biomarkers might be potential biomarkers for human aging and type 2 diabetes.

The current study investigated a number of factors that may affect telomere attrition in the analysis and confirmed the previous findings that shorter telomeres and increased serological N-acetyl-glucosaminidase levels correlate with aging. More importantly, this study is the first to identify that serological N-acetyl-glucosaminidase represents an independent risk factor for type 2 diabetes.

Acknowledgments—This work was supported by the National Science and Technology Pillar Program (2007BAI24B08) and the National Natural Science Foundation of China (81130074). Z.J. is supported by the MPG Partner Group Program on Stem Cell Aging.

No potential conflicts of interest relevant to this article were reported.

F.X., X.Z., M.C., G.S., X.C., and R.L. researched data. B.C. and Z.J. wrote the manuscript and researched data. Z.S. and K.L.R. edited the manuscript and contributed to the discussion.

The authors acknowledge the editorial assistance of Hantz C. Hercule, Charite University of Medicine, Berlin, Germany.

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