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Association of telomere length with type 2 diabetes, oxidative stress and *UCP2* gene variation

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ABSTRACT

Objective: High oxidative stress potentially leads to accelerated telomere shortening and consequent premature cell senescence, implicated in type 2 diabetes (T2D) development. Therefore, we studied the association of leukocyte telomere length (LTL) with the presence of T2D, as well as the effect on the patients' LTL of plasma oxidative stress and of variation in *UCP2,* a gene involved in the mitochondrial production of reactive oxygen species.
Methods: Mean LTL was determined in 569 Caucasian, 103 South Asian and 70 Afro-Caribbean T2D patients aged from 24 to 92 years, 81 healthy Caucasian male students aged from 18 to 28 years and 367 healthy Caucasian men aged from 40 to 61 years by real-time PCR. Plasma total antioxidant status (TAOS) was measured in the T2D patients by a photometric microassay. The patients were also genotyped for the *UCP2* functional variants −866G>A and A55V.
Results: Afro-Carribeans had 510 bp longer mean length compared to Caucasians ($p < 0.0001$) and 500 bp longer than South Asians ($p = 0.004$). T2D subjects displayed shorter age-adjusted LTL compared to controls [6.94(6.8–7.03) vs. 7.72(7.53–7.9), $p < 0.001$] with subjects in the middle and the lowest tertile of LTL having significantly higher odds ratios for T2D compared to those in the highest tertile [1.50(1.08–2.07) and 5.04(3.63–6.99), respectively, $p < 0.0001$]. In the patients, LTL was correlated negatively with age ($r = -0.18$, $p < 0.0001$) and positively with TAOS measures ($r = 0.12$, $p = 0.01$) after adjusting for age, while carriers of the *UCP2* −866A allele had shorter age-adjusted LTL than common homozygotes [6.86(6.76–6.96) kb vs. 7.03(6.91–7.15) kb, $p = 0.04$].
Conclusion: The present data suggest that shorter LTL is associated with the presence of T2D and this could be partially attributed to the high oxidative stress in these patients. The association of the *UCP2* functional promoter variant with the LTL implies a link between mitochondrial production of reactive oxygen species and shorter telomere length in T2D.

1. Introduction

Premature cell senescence has recently been postulated as an important cause and consequence of type 2 diabetes (T2D) and its complications [1]. The telomere shortening hypothesis is a widely accepted mechanism leading to senescence [2]. Telomeres are specialised DNA-protein structures at the end of all chromosomes, which preserve chromosome stability and integrity. In humans they consist of thousands of tandem repeats of the TTAGGG sequence [3]. Telomeres shorten with cell division and cells are triggered into senescence once mean length reduces below a critical value [2].

Telomere attrition is mainly caused by the "end-replication" problem, generated by the incapability of DNA polymerase to fully copy the very end of the lagging strand [4]. Another factor contributing to telomere attrition involves the processing of telomere ends to reconstitute 3′ single-strand overhangs, and telomere loss due to the fact that DNA repair mechanisms, particularly for single-stranded DNA damage, are less efficient in telomeric DNA than elsewhere in the genome. The resulting accumulation of single-strand breaks along the telomeres leads to DNA damage-dependent shortening during replication [5]. Hence, telomere shortening could serve as an indicator of replicative history and cumulative genomic damage of somatic cells. Telomeric DNA is particularly prone to oxidative damage at the GGG sequence. Exposure to free

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radicals or oxidants causes DNA damage including single-strand breaks and telomere erosion as shown with *in vitro* experiments [6–8]. Therefore, it is speculated that the rate of telomere shortening will be dependent on the balance between intracellular oxidative stress and antioxidant defence.

Prediabetes and metabolic syndrome are associated with increased oxidative stress [9,10]. It is well established that hyperglycaemia elicits an increase in reactive oxygen species (ROS) production, due to increased input of reducing equivalents into the mitochondrial electron transport chain. ROS overproduction is a trigger for pathways responsible for hyperglycaemia-induced cell damage [11,12].

Our hypothesis is that shorter telomeres eventually lead to senescent phenotypes in multiple cell types including beta cells, the consequent apoptosis of which hastens the onset of diabetes. These shorter telomeres can be either attributed to shorter length at birth in individuals predisposed to diabetes *or* to accelerated telomere loss during cell division caused by increased oxidative stress in prediabetic conditions, *or* both. In support of this, shorter telomeres have been observed in circulating epithelial progenitor cells in patients with metabolic syndrome [13] and in other conditions of high oxidative stress, such as smoking and obesity [14]. The data on T2D and telomere length though are scarce with only few small studies with up to 80 subjects showing that T2D patients have shorter telomeres than controls [15–17]. Whether this is due to oxidative stress, and to what degree, remains to be determined. Therefore, our aim was to examine the association of telomere length with the presence of T2D in a large cohort as well as the effect of plasma oxidative stress on the patients' leukocyte telomere length.

In order to further enlighten the effect of ROS on telomere shortening, we also studied the effect of uncoupling protein 2 (*UCP2*) gene variation. This ubiquitously expressed protein is a plausible negative regulator of ROS production, since it dissipates the inner mitochondrial membrane electrochemical gradient that drives ATP synthesis and uncouples respiration from oxidative phosphorylation [18]. Decreased *UCP2* expression results in increased ROS production, *in vitro* [19], while animal studies have shown that absence of *UCP2* causes higher oxidative stress [20]. To maintain homeostasis, *UCP2* expression is induced by elevated oxygen species concentration [21]. A common functional variant exists in the promoter of human *UCP2* gene (−866G>A), with the A allele being associated with lower mRNA levels, while a non-synonymous SNP leading to an alanine to valine substitution has been indentified in exon 4 of the gene (A55V) [22]. A previous study from our laboratory, demonstrated that the *UCP2* −866G>A variant interacts with smoking to increase oxidative stress in T2D patients [23]. Given this finding and the established function of *UCP2*, we hypothesised that these functional variants in the *UCP2* gene will be associated with the stress-induced telomeric DNA damage and therefore with the telomere length of T2D patients, in whom oxidative stress is elevated.

2. Materials and methods

2.1. Subjects

2.1.1. University College London Diabetes and Cardiovascular disease Study (UDACS)

The UDACS is a cross-sectional sample of diabetes patients, according to the World Health Organization criteria [24], designed to study the association between common genetic variants and biochemical risk factors implicated in coronary heart disease (CHD) in patients with diabetes. It comprises of 1011 subjects consecutively recruited from the diabetes clinic at UCL Hospitals in 2001–2. Analyses were confined only to T2D patients ($N = 742$). Ethical approval was obtained from UCL/UCL Hospitals ethics committee and all subjects gave informed consent. The full characteristics of patients have been reported previously [25].

2.1.2. European Atherosclerosis Research Study II (EARS II)

The EARSII was carried out in 1993. Male students between the ages of 18 and 28 years whose fathers had a proven myocardial infarction before the age of 55 (cases, $N = 407$) and age-matched male controls ($N = 415$) were recruited from 14 university student populations of 11 European countries: Tallinn in Estonia, Helsinki and Oulu in Finland were designated Baltic; Glasgow, Belfast and Bristol were designated United Kingdom (UK); Aarhus in Denmark, Hamburg in Germany, Ghent in Belgium, and Zurich in Switzerland were designated Middle Europe; Lisbon in Portugal, Reus in Spain, Naples in Italy, and Athens in Greece were designated South Europe. Only the controls were considered in the present study. The study has been approved by ethics committees of collaborating centres and the subjects have given informed consent. Details of the study have been described previously [26].

2.1.3. Hypercoagulability and Impaired Fibrinolytic function MECHanisms predisposing to myocardial infarction (HIFMECH)

The HIFMECH study consists of 598 male survivors of a first myocardial infarction aged <60 years (excluding patients with familial hypercholesterolaemia and insulin-dependent diabetes mellitus) and 653 population-based control subjects of the same age and region recruited from four centers in Europe: Stockholm in Sweden and London in England were designated North Europe and Marseille in France and San Giovanni Retondo in Italy were designated South Europe. The study has been approved by ethics committees of collaborating centres and the subjects have given informed consent. Detailed description of the study can be found elsewhere [27].

2.2. Determination of plasma total antioxidant status (TAOS)

The UDACS plasma samples were collected within a 12-month period and stored immediately at −80 °C. A modification by Sampson of Laight's photometric microassay [28] was used to measure plasma TAOS. In this method, plasma TAOS is determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbensthiazoline-6-sulfonic acid radical. The assay is performed in a 96-well ELISA plate with 2.5 μL of plasma. The inter-assay coefficient of variation was 14% and the intra-assay 4.3%. In general terms, increased oxidative stress within a sample is negatively correlated with consumption of antioxidants and diminished antioxidant status within that sample.

In order to test whether TAOS is a valid measure of oxidative stress, we have previously examined the correlation between plasma TAOS and esterified F2-isoprostane and found this to be significant ($r = -0.65$; $p = 0.003$) [29]. Since the measures obtain by the two methods correlate, the inter-individual differences in TAOS and/or the correlation to other variables is feasible by using either method.

2.3. Determination of leukocyte telomere length

Leukocyte DNA was extracted by the salting-out method [30]. Telomere length was measured in these DNA samples using a validated quantitative PCR-based method as previously described [26]. Briefly, the relative telomere length was calculated as the ratio of telomere repeats to single-copy gene (SCG) copies (T/S ratio). For each sample the quantity of telomere repeats and the quantity of SCG copies were determined in comparison to a reference sample in a telomere and a SCG quantitative PCR, respectively. The raw