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Telomere shortening occurs in Asian Indian Type 2 diabetic patients

A. Adaikalakoteswari, M. Balasubramanyam and V. Mohan

Department of Cell and Molecular Biology, Madras Diabetes Research Foundation, Chennai, India

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Abstract

Aim Telomere shortening has been reported in several diseases including atherosclerosis and Type 1 diabetes. Asian Indians have an increased predilection for Type 2 diabetes and premature coronary artery disease. The aim of this study was to determine whether telomeric shortening occurs in Asian Indian Type 2 diabetic patients.

Methods Using Southern-blot analysis we determined mean terminal restriction fragment (TRF) length, a measure of average telomere size, in leucocyte DNA. Type 2 diabetic patients without any diabetes-related complications ($n = 40$) and age- and sex-matched control non-diabetic subjects ($n = 40$) were selected from the Chennai Urban Rural Epidemiology Study (CURES). Plasma level of malondialdehyde (MDA), a marker of lipid peroxidation, was measured by TBARS (thiobarbituric acid reactive substances) using a fluorescence method.

Results Mean (± SE) TRF lengths of the Type 2 diabetic patients (6.01 ± 0.2 kb) were significantly shorter than those of the control subjects (9.11 ± 0.6 kb) ($P = 0.0001$). Among the biochemical parameters, only levels of TBARS showed a negative correlation with shortened telomeres in the diabetic subjects ($r = -0.36$; $P = 0.02$). However, telomere lengths were negatively correlated with insulin resistance (HOMA-IR) ($r = -0.4$; $P = 0.01$) and age ($r = -0.3$; $P = 0.058$) and positively correlated with HDL levels ($r = 0.4$; $P = 0.01$) in the control subjects. Multiple linear regression (MLR) analysis revealed diabetes to be significantly ($P < 0.0001$) associated with shortening of TRF lengths.

Conclusions Telomere shortening occurs in Asian Indian Type 2 diabetic patients.

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Keywords Telomere, diabetes mellitus, oxidative stress, senescence, Asian Indians

Introduction

Telomeres, the TTAGGG tandem repeats at the ends of mammalian chromosomes, undergo attrition with each division of somatic cells in culture, and hence their length is an indicator of the replicative potential of these cells [1]. The inability of DNA polymerases to replicate a linear DNA molecule to its very end [2] and the action of a strand-specific exonuclease [3] are believed to contribute to the shortening of telomeres. Increased oxygen tension has also been shown to accelerate telomere shortening in replicating fibroblasts *in vitro* [4]. Telomeric DNA sequences appear to be particularly prone to chromosomal breakage [5], and their GGG-triplets are a major target for reactive oxygen species [6–8].

Recent studies have demonstrated that telomere shortening is related to various pathological conditions including atherosclerosis [9–12]. Jeanclos *et al*. [13] established an association between telomere shortening in white blood cells (WBCs) and Type 1, but not in Type 2, diabetic patients of European origin. Asian Indian Type 2 diabetic patients differ from Europeans in several aspects: the onset of diabetes occurs at a younger age [14], and there is a greater degree of hyperinsulinaemia [15] and insulin resistance [16]. In addition they have very high prevalence rates of premature coronary artery disease [17,18].

Correspondence to: Dr M. Balasubramanyam, Department of Cell and Molecular Biology, Madras Diabetes Research Foundation, 4, Conran Smith Road, Gopalapuram, Chennai—600 086, India. E-mail: drbalu@mvdsc.org

In this paper we report that telomere lengths are shortened in urban southern Indian Type 2 diabetic patients. This is the first report, to our knowledge, of telomere shortening in Type 2 diabetes.

Subjects and methods

Sample selection

The Chennai Urban Rural Epidemiology Study (CURES) is an ongoing epidemiological study conducted on a representative population of Chennai (formerly Madras), the fourth largest city in India with a population of approximately 4.2 million. The methodology of the study has been published elsewhere [19,20]. Briefly, in Phase 1 of the urban component of CURES, 26 001 individuals were recruited based on a systematic random sampling technique. Self-reported diabetic subjects were classified as 'known diabetic subjects'.

In Phase 2 of CURES, all known diabetic subjects (n = 1529) were invited to our centre for detailed studies on vascular complications. In addition, age- and sex-matched non-diabetic subjects underwent oral glucose tolerance tests (OGTT) using 75 g of glucose load. Those who were confirmed by OGTT to have fasting venous plasma glucose < 6.1 mmol/l and a 2-h plasma glucose value < 7.8 mmol/l were categorized as normal glucose tolerance (NGT). For the present study we randomly selected 40 diabetic subjects without any complications (using computer-generated random numbers) and 40 age- and sex-matched subjects with NGT. The study had a power of 80% to detect a statistically significant (P = 0.05) difference of 2.0 kb in TRF between the two study groups.

Physical examination included height, weight, waist and hip measurements using standardized techniques. Blood pressure was recorded in the right arm with a mercury sphygmomanometer (Diamond Deluxe Blood Pressure Apparatus, Pune, India) while the patients were seated. Two readings were taken 5 min apart and the mean of the two was taken as the blood pressure. A fasting blood sample was taken, and serum separated and stored at –70°C until the assays were performed. Biochemical analyses were carried out on a Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). Fasting plasma glucose (GOD-POD method), serum cholesterol (CHOD-PAP method), serum triglycerides (GPO-PAP method) and HDL cholesterol (direct method—polyethylene glycol-pretreated enzymes) were measured. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [21]. Glycated haemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA, USA). Serum insulin concentration was estimated using Dako kits (Dako, Glostrup, Denmark). Insulin resistance (HOMA-IR) was calculated using the Homeostasis Model Assessment using the formula: fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5 [22]. Informed consent was obtained from all study subjects, and the study was approved by the institutional Ethics Committee.

To avoid the confounding effect of diabetic complications on telomeric shortening, the diabetic subjects selected had no evidence of retinopathy (assessed by retinal photography) or nephropathy (24-h protein excretion < 100 mg/day and urinary albumin levels < 30 μg/mg creatinine). They also had no history of angina or myocardial infarction, and a normal 12-lead resting ECG. Hypertension was diagnosed if the subjects had been treated with antihypertensive drugs or had systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg.

TRF length analysis

Genomic DNA was prepared from whole blood by digestion with proteinase-K and extraction with phenol/chloroform and was quantified spectrophotometrically. The DNA samples extracted from WBCs were coded, and only after completion of the TRF measurement was the code broken for data analysis. Terminal restriction fragment (TRF) lengths were measured using the Southern-blotting technique [13]. Briefly, equal amounts of DNA (2 μg) were digested with restriction enzymes HinfI (20 U) and RsaI (20 U) (Roche Diagnostics) for 2 h at 37°C to liberate TRFs, which include both subtelomeric repetitive DNA and telomeric TTAGGG repeats. The TRFs that determines the telomere lengths were separated by electrophoresis on 0.8% agarose gel denatured with 0.5 M NaOH/1.5 M NaCl and neutralized for 30 min in 0.5 M Tris and 1.5 M NaCl. The DNA was transferred overnight to a nylon membrane positively charged using capillary transfer. The membranes were then hybridized with telomeric probe digoxigenin 3′end labelled 5′-$(CCCTAA)_3$ for 3 h in the hybridization solution. They were then washed at room temperature, three times in 2 × saline sodium citrate (SSC), 0.1% SDS each for 15 min and once in 2 × SSC for 15 min. The digoxigenin-labelled probe was detected by the digoxigenin luminescent detection procedure and exposed on X-ray film. The mean TRF length was determined using automated BIORAD Gel documentation software and calculated as follows: TRF = Σ ODi/(Σ ODi/Σ MWi), where ODi is the optical density at a given position in the lane and MWi is the molecular weight at that position; this formula [23] accounts for the fact that longer telomeres bind more labelled probes and consequently appear darker on the X-ray film (Fig. 1). Proper reference standards and molecular-weight markers were included in all blots. To show the reproducibility of our method, we measured the telomere lengths of eight subjects on two different occasions. For this, blood samples were taken twice from the same subject on two different occasions and the respective DNA used for the TRF length measurements were referred to as TRF1 and TRF2. As shown in Fig. 2, the two values correlated well (r = 0.93; P < 0.001), indicating that white blood cell mean TRF length is a reproducible measure. The interassay coefficient of variation was < 3% and the maximum difference between the two blots carried out from the same subject on different days (blot-to-blot variability) was < 7%, i.e. < 0.5 kb.

Lipid peroxidation

Plasma levels of malondialdehyde (MDA), a marker of lipid peroxidation, were measured by TBARS (thiobarbituric acid reactive substances) by fluorescence methodology [24]. Plasma (200 μl) was treated with 8.1% SDS and 20% acetic acid to solubilize and precipitate protein and then heated with TBA for 1 h at 95°C. The supernatant was then extracted with butanol:pyridine (15 : 1), to produce a fluorescent product, which

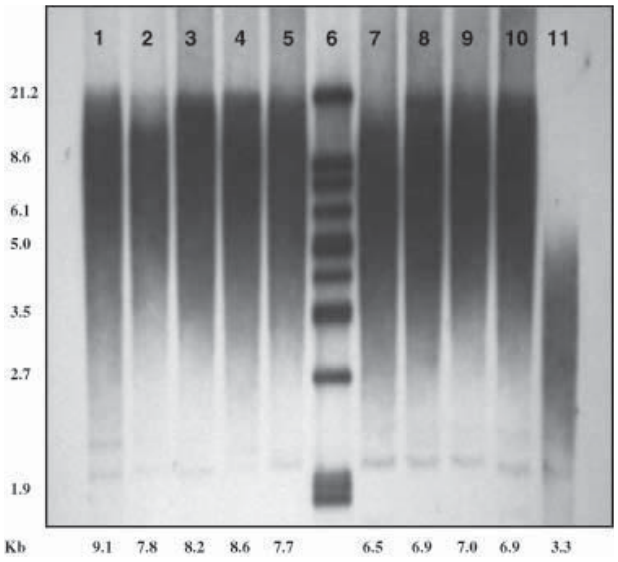

Figure 1 Autoradiograph showing the lengths of the terminal restriction fragment (TRF) of genomic DNA from white blood cells (WBCs). Lanes 2–5: samples from the control subjects. Lanes 7–10: samples from the diabetic subjects; Lanes 1 & 11: reference samples (High MWt & Low MWt); Lane 6: molecular weight marker (1.9–21.2 Kb).

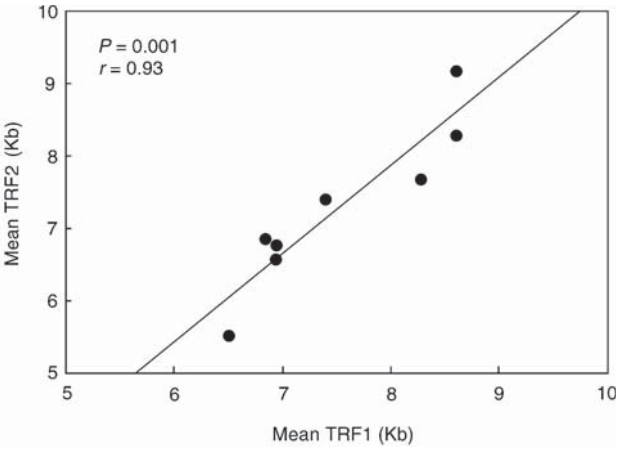

Figure 2 Reproducibility of mean telomere restriction fragment (TRF) length assay. Blood samples were taken twice from the same subject on two different occasions and the respective DNA was used for the determination of telomere lengths (referred as TRF1 and TRF2). Excellent correlation ($r = 0.93$; $P = 0.001$) between these two measurements obtained from eight individuals.

was detected by excitation at 535 nm and emission at 553 nm. Absolute MDA (malonodialdehyde) levels were calculated using the regression parameters obtained using various concentrations (0.25–5.0 nM) of the standard, 1,1′,3,3′,-tetramethoxypropane. Inter- and intra-assay coefficients of variation of the above method were < 5 and 10%, respectively.

Statistical analysis

Comparisons between groups were performed using an unpaired Student's *t*-test. Two-tailed *P*-values equal to or less than 0.05 were considered statistically significant. Pearson correlation analysis was performed between variables. Risk variables that had a significant association with TRF on univariate regression were included as independent variables in multiple linear regression analysis. Care was taken to avoid intercorrelated variables in the regression equation. All analyses were carried out using a Windows-based SPSS statistical package (Version 10.0, Chicago, IL, USA).

Results

Table 1 shows the characteristics of both the diabetic and non-diabetic subjects. None of the diabetic patients had ketonuria or any history of diabetic ketosis at any time and all were treated with oral agents sulphonylurea (glipizide or glibenclamide) and/or metformin. Hence they all had Type 2 diabetes. Diabetic patients had significantly higher plasma glucose, HbA_{1c}, serum cholesterol, triglycerides and HOMA-IR compared with the control subjects.

The mean (± SE) TRF lengths were significantly lower in the patients with Type 2 diabetes (6.01 ± 0.2; range 3.0–9.5 kb) compared with the control subjects (9.11 ± 0.6; range 3.5–15.8 kb) ($P = 0.0001$) (Fig. 3). Age-adjusted telomere lengths were significantly shorter in men (7.69 ± 0.6) than in women (10.42 ± 1.0) ($P = 0.02$) in the control subjects. This gender difference in TRF length was not observed among the diabetic subjects (men 5.81 ± 0.4, women 6.22 ± 0.3, $P = 0.51$). When compared with the diabetic subjects without hypertension (6.74 ± 0.3) the diabetic subjects with hypertension exhibited significantly shorter TRF lengths (5.23 ± 0.4) ($P = 0.007$).

Lipid peroxidation as measured by TBARS was significantly higher in the patients with Type 2 diabetes (9.69 ± 1.0 nM/ml) when compared with the control subjects (6.17 ± 0.3 nM/ml) (Fig. 4a). Increased levels of TBARS also showed a negative correlation with shortened telomeres in the diabetic subjects ($r = -0.36$; $P = 0.02$) (Fig. 4b). However, such a relationship

Table 1 Clinical characteristics of the study subjects

Parameters	Control ($n = 40$)	Type 2 diabetes ($n = 40$)
Age (years)	49 ± 8	49 ± 7
Male: Female (n)	20 : 20	20 : 20
Duration of diabetes (years)	—	3.3 ± 2.8
Body mass index (kg/m^2)	23.5 ± 4.2	25.2 ± 3.8
Fasting plasma glucose (mmol/l)	4.7 ± 0.8	8.3 ± 3.5*
HbA_{1c} (%)	5.5 ± 0.6	8.7 ± 2.6*
Systolic blood pressure (mmHg)	126 ± 19	125 ± 25
Diastolic blood pressure (mmHg)	77 ± 11	79 ± 13
Serum Cholesterol (mmol/l)	4.6 ± 0.8	5.1 ± 1.1*
Serum Triglycerides (mmol/l)	1.4 ± 0.6	2.1 ± 1.1*
Serum HDL cholesterol (mmol/l)	1.08 ± 0.3	1.05 ± 0.2
Serum LDL cholesterol (mmol/l)	2.9 ± 0.7	3.1 ± 0.9
HOMA-IR	1.75 ± 1.1	4.04 ± 2.9*

Values are expressed as mean ± SD.
*$P < 0.05$ compared with control.

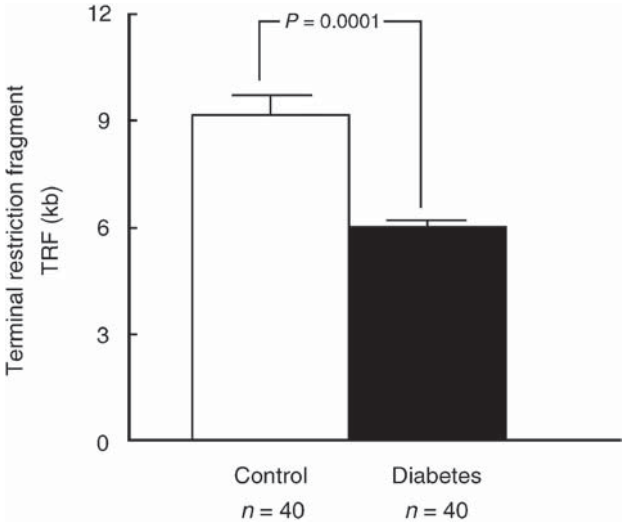

Figure 3 Mean (± SE) telomere lengths of the control (9.11 ± 0.6 kb) and Type 2 diabetic subjects (6.01 ± 0.2 kb).

between TBARS and TRF was not observed in the control group.

Telomere lengths were negatively correlated with insulin resistance (HOMA-IR) ($r = -0.4$; $P = 0.01$) and age ($r = -0.3$; $P = 0.058$) and positively correlated with HDL levels ($r = 0.4$; $P = 0.01$) in the control subjects. Correlation of TRF lengths with HbA_{1c} levels ($r = -0.28$; $P = 0.01$) and cholesterol to HDL ratio ($r = -0.31$; $P = 0.005$) was obvious only in the total study subjects. Multiple linear regression analysis carried out using diabetes status, cholesterol to HDL ratio and TBARS as independent variables revealed the presence of diabetes as the sole risk factor associated with telomere shortening ($\beta = -2.71$, $P < 0.0001$).

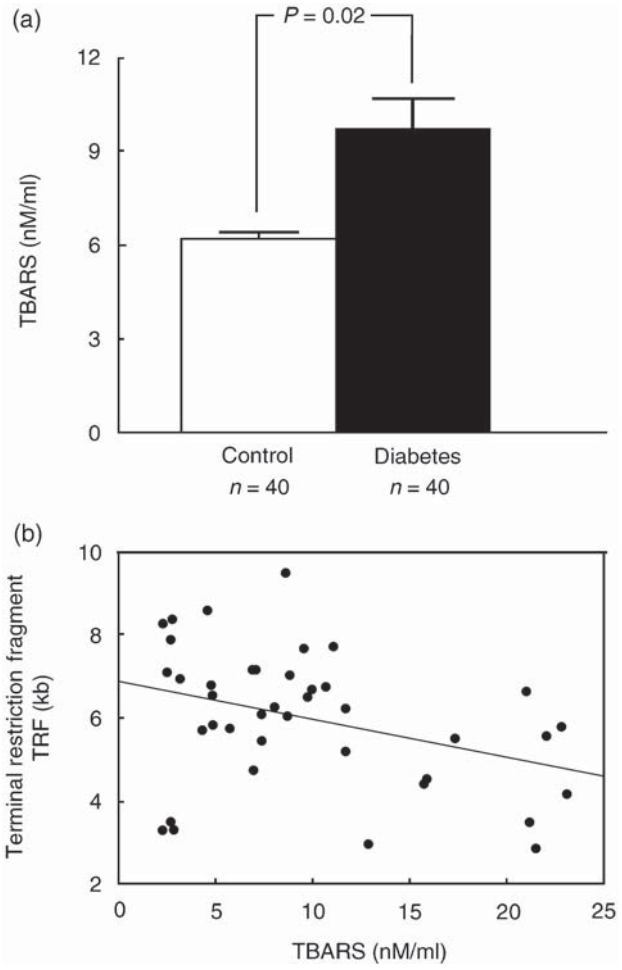

Figure 4 (a) Mean (± SE) lipid peroxidation levels in the Type 2 diabetes (9.69 ± 1.0 nM/ml) and control subjects (6.17 ± 0.3 nM/ml). (b) Correlation between TBARS (thiobarbituric acid reactive substances) and terminal restriction fragment (TRF) length in the diabetic subjects ($r = -0.36$; $P = 0.02$).

Discussion

Shortening of telomeres has been reported to be present in patients with inherited respiratory chain disorders [25], Down's syndrome [26], vascular dementia [27] and ataxia-telangiectasia [28]. Recent studies have also shown that telomere shortening can be a biomarker of premature cell senescence in vascular diseases and metabolic disorders [10,11,12].

An earlier study from the USA demonstrated telomere shortening in Type 1, but not in Type 2 diabetic patients of European descent [13]. Our study is the first to demonstrate a shortened TRF length in WBCs in patients with Type 2 diabetes. It is possible that the increased insulin resistance observed in Asian Indians [16] contributes to the telomere shortening observed in our patients. This is supported by the association of the telomere shortening with HOMA-IR observed in this study. Our results also indicate the presence of diabetes as one of the risk factors associated with telomere shortening. It is possible that chronic hyperglycaemia and associated oxidative stress may modify TRF length in WBCs. Alternatively, a diminished TRF length could be a secondary phenomenon arising out of accelerated telomere attrition owing to an increased turnover and chronic activation of inflammatory cells.

In non-diabetic subjects, the age-adjusted telomere length is shorter in men than in women, confirming earlier studies [29–31]. As premenopausal women are less prone than men to cardiovascular diseases [32] and several systemic parameters show poor correlation with blood pressure in women [33], our observations suggest that the biology of ageing differs between men and women. Moreover, an oestrogen-responsive element is present in hTERT [34], and hence hormonal changes may influence telomerase to maintain the telomere length in women. However, this gender difference was absent in Type 2 diabetic subjects. It is well known that women with Type 2 diabetes lose their protection from coronary artery disease [35]. Our study confirms these findings using TRF as a marker.

Within the diabetic subjects, those with hypertension also showed a significantly reduced TRF length when compared with subjects without hypertension. Hypertensive subjects are

at higher risk for atherosclerosis and accelerated cardiovascular ageing [30]. Increased oxidative stress has been considered as one of the molecular determinants of cardiovascular diseases [36]. Therefore telomere length may provide an additional link between oxidative stress and the predisposition to cardiovascular disease in hypertensive subjects.

The association of short telomeres with insulin resistance (as measured by HOMA-IR) and cholesterol to HDL ratio suggests that telomere shortening could probably be used as an additional marker of atherosclerosis. In support of this, coronary artery disease patients have been shown to exhibit telomere shortening [11]. Asian Indians have high prevalence rates of premature coronary artery disease [17,18,37]. The excess risk for coronary artery disease seen among Asian Indians has hitherto not been explained by any of the conventional risk factors. From this study one could speculate that telomere attrition could be one of the molecular mechanisms that predispose Asian Indian diabetic patients to premature coronary artery disease. However, further work needs to be performed on diabetic patients with and without coronary artery disease to confirm this hypothesis. It is also known that several additional factors such as oxygen-free radicals and elevated plasma homocysteine, which are overproduced in some metabolic diseases, are responsible for DNA damage and telomere shortening, and can induce atherosclerosis [8,38–40]. The positive association of HDL cholesterol and TRF length in our study also lends further support to this hypothesis. Interestingly it was recently shown that increased HDL cholesterol and TRF were linked to increased longevity even in animal models [41–43].

The association of increased lipid peroxidation (TBARS) with shorter telomeres in our study may indicate a role for reactive oxygen species in telomere shortening. Schisterman *et al*. [44] have shown that individuals with coronary heart disease had significantly higher levels of TBARS and lipid peroxidation was found to be the best discriminator when the biomarkers of oxidative stress were evaluated individually. Studies in cultured cells have shown that telomere erosion per replication is inversely related to antioxidant capacity [4,45,46]. Inflammation and ROS thus appear to be important factors in the pathobiology of age-related disorders, including Type 2 diabetes [47,48]. As suggested by Furumoto *et al*. [49] telomere length registers the turnover rate of cells, including WBC, a rate that may be augmented by chronic inflammation, and an increase in the cumulative oxidative stress. We found a good correlation between TBARS and telomere shortening. However, in our multiple linear regression model diabetes was the only significant factor in determining telomere shortening, and addition of TBARS did not affect this relationship. This may be explained by the fact that in diabetes, oxidative stress appears as an early biochemical defect and thus further addition of TBARS into the model only had minimal influence.

In summary, we report that telomere shortening of WBCs is observed in Asian Indian patients with Type 2 diabetes. Oxidative stress could be a common molecular mechanism in which the expression of genes related to glucose metabolism, lipid metabolism and vascular function are modified in subjects with diabetes. As telomere length is also highly heritable [29,50], and probably X-linked in some cases [31], the role of genetic predisposition to shortened telomeres in chronic age-related disorders needs further investigation. Telomeres shorten very slowly with age, raising the exciting possibility that telomere shortening may be a risk marker of diabetes and its vascular complications. Future studies are needed to look at telomere shortening in diabetic micro- and macro-vascular complications, and also into whether the shortening can be reversed, at least in part, by tight control of diabetes.

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Linking a role of lncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes

Chandrakumar Sathishkumar, Paramasivam Prabu, Viswanathan Mohan and Muthuswamy Balasubramanyam*

Abstract

Background: Studying epigenetics is expected to provide precious information on how environmental factors contribute to type 2 diabetes mellitus (T2DM) at the genomic level. With the progress of the whole-genome resequencing efforts, it is now known that 75–90% of the human genome was transcribed to generate a series of long non-coding RNAs (lncRNAs). While lncRNAs are gaining widespread attention as potential and robust biomarkers in the genesis as well as progression of several disease states, their clinical relevance and regulatory mechanisms are yet to be explored in the field of metabolic disorders including diabetes. Despite the fact that Asian Indians are highly insulin resistant and more prone to develop T2DM and associated vascular complications, there is virtually lack of data on the role of lncRNAs in the clinical diabetes setting. Therefore, we sought to evaluate a panel of lncRNAs and senescence-inflammation signatures in peripheral blood mononuclear cells (PBMCs) from patients with type 2 diabetes (T2DM; $n = 30$) compared to individuals with normal glucose tolerance (NGT; $n = 32$).

Results: Compared to control subjects, expression levels of lncRNAs in PBMCs from type 2 diabetes patients showed significantly ($p < 0.05$) increased levels of HOTAIR, MEG3, LET, MALAT1, MIAT, CDKN2BAS1/ANRIL, XIST, PANDA, GAS5, Linc-p21, ENST00000550337.1, PLUTO, and NBR2. In contrast, lncRNA expression patterns of THRIL and SALRNA1 were significantly ($p < 0.05$) decreased in patients with T2DM compared to control subjects. At the transcriptional level, senescence markers (p53, p21, p16, and β-galactosidase), proinflammatory markers (TNF-α, IL6, MCP1, and IL1-β), and epigenetic signature of histone deacetylase-3 (HDAC3) were significantly ($p < 0.05$) elevated in patients with type 2 diabetes compared to control subjects. Interestingly, mRNA expression of Sirt1 and telomere length were significantly ($p < 0.05$) decreased in patients with type 2 diabetes compared to control subjects. Majority of the altered lncRNAs were positively correlated with poor glycemic control, insulin resistance, transcriptional markers of senescence, inflammation, and HDAC3 and negatively correlated with telomere length. Logistic regression analysis revealed a significant association of altered lncRNA signatures with T2DM, but this association was lost after adjusting for insulin resistance (HOMA-IR) and senescence markers.

Conclusion: Our study provides a clinically relevant evidence for the association of altered lncRNAs with poor glycemic control, insulin resistance, accelerated cellular senescence, and inflammation.

Keywords: lncRNA, SASP, HDAC3, Type 2 diabetes, Insulin resistance, Inflammation

* Correspondence: balusignal@gmail.com
Department of Cell and Molecular Biology and Dr. Rema Mohan High-Throughput Screening (HTS) Lab, Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialties Centre, Gopalapuram, Chennai 600 086, India

Introduction

According to the latest edition of International Diabetes Federation Atlas [1], around 425 million people worldwide have diabetes and India alone harbors more than 73 millions of people with diabetes. While more than 90% of the diabetic population is affected majorly by type 2 diabetes mellitus (T2DM), it is a complex multi-factorial disease involving genetic, epigenetic, and environmental components. Several studies imply that accelerated aging, cellular senescence, and proinflammation are closely linked to the etiology of type 2 diabetes and insulin resistance [2, 3]. Although the association between the proinflammation and senescence in the development of insulin resistance and type 2 diabetes is well known, the underlying molecular mechanisms and upstream regulatory networks are only poorly understood. Epigenetics appears to play a major role in the regulation of inflammation and cellular senescence—the dual pathological features commonly associated with type 2 diabetes [4]. Aberrant epigenetic modifications such as DNA methylation, histone modification, and non-coding RNA alterations are well-recognized drivers for the cancer phenotype, but the accumulating evidence also implies their role in the etiology of diabetes and cardiovascular diseases.

Of the total genome that is transcribed, only 2% codes for proteins, whereas the vast majority of it is transcribed as non-coding RNAs which include long non-coding RNAs (lncRNAs), microRNAs, and others [5]. Of late, lncRNAs have gradually come into the spotlight for the increased appreciation of their functional importance both in health and disease [6]. lncRNAs were also found next to protein-coding genes that are under tight transcriptional control, and often, their expression pattern correlates with tissue differentiation, development, and disease [7]. The widespread dysregulation of lncRNA expression in several disease states and the finding that many lncRNAs are enriched for SNPs that associate with human traits/diseases have highlighted their role as master regulators [8, 9]. Challenging the concept that protein-coding genes are the sole contributors to the development of human disease, recent studies emphasize that lncRNAs mediate disease pathogenesis and hence should be studied and targeted for therapeutic benefits [10]. Accumulating literature on genetic, experimental, and epidemiological studies also highlights a growing list of lncRNAs that control glucose homeostasis and contribute to the pathogenesis of diabetes and its complications. Despite the fact that Asian Indians are highly insulin resistant [11] and more prone to develop T2DM and associated vascular complications [12], there is lack of data on the role of lncRNAs in the clinical diabetes setting and this is the rationale behind our study. Therefore, we planned to study the potential interactions among insulin resistance, cellular senescence, and proinflammation with a central focus on lncRNAs so as to better understand the clinical significance of these molecular perturbations in type 2 diabetes.

Research design and methods

Recruitment of the study subjects

Study participants with normal glucose tolerance (NGT; $n = 32$) and patients with type 2 diabetes (T2DM; $n = 30$) were recruited from Dr. Mohan's Diabetes Specialties Centre, Chennai, India, and from the ongoing epidemiological cohorts. The study was approved by the institutional ethics committee of the Madras Diabetes Research Foundation and conducted according to the principles of Declaration of Helsinki. Written informed consent was obtained from all the study participants prior to the start of the study. All the study participants were clinically well characterized into respective groups according to the World Health Organization (WHO) classification criteria. While all the diabetic patients were on oral hypoglycemic agent (OHA) treatment, < 10% were also on insulin, in addition to OHA.

Anthropometric measurements

Anthropometric measurements including weight, height, and waist circumference were obtained using standardized techniques. Height was noted down with a tape measured to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Body mass index (BMI) was calculated using the formula: weight (kg)/height (m^2). Waist circumference was measured using a non-stretchable fiber measuring tape. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mmHg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart, and the mean of the two readings was represented as the blood pressure.

Biochemical and clinical investigations

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method), and HDL cholesterol (direct method–polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra and inter assay co-efficient of variation for the biochemical assays was < 5%. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [13]. Glycated hemoglobin (HbAlc) was estimated by high-pressure liquid chromatography using the variant analyzer (Bio-Rad, Hercules, Calif., USA). Serum insulin was estimated using enzyme-linked immunosorbent assay (Calbiotech, CA). Insulin resistance was calculated using the homeostasis

assessment model (HOMA-IR) using the formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5.

Blood collection and isolation of peripheral blood mononuclear cells (PBMCs)

Fasting blood (5–8 mL) was collected into the vacutainer tube and processed immediately for cell isolation within 2 h from the time of collection. Blood was processed for peripheral blood mononuclear cell (PBMC) isolation using Histopaque-1077 (Sigma-Aldrich) according to the standard protocol by overlaying the blood on density gradient solution and centrifugation at 1500–1800 rpm for 30 min. The buffy coat layer containing the PBMCs was aspirated, washed thrice with phosphate-buffered saline (PBS; pH 7.2–7.4), and aliquoted for various experiments.

RNA extraction and cDNA synthesis

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA quantity and quality were assessed by NanoDrop 2000 (Thermo Scientific) instrument. For the first-strand cDNA synthesis reaction, total RNA (1 μg) was adjusted with nuclease-free water and mixed with the cDNA synthesis master mix containing 100 units of RevertAid M-MuLV reverse transcriptase enzyme and 2× buffer, random hexamer primers (1×), 20 units of RNase inhibitor, and 10 mM dNTP solution mix. The resultant samples were incubated at 42 °C for 60 min for the first-strand cDNA synthesis followed by a 5-min incubation at 95 °C for enzyme deactivation. cDNA reaction negative control without reverse transcriptase enzyme (−RT) was also performed.

lncRNA/mRNA expression by Q-PCR

A panel of lncRNAs was chosen for this study based on their involvement in metabolic disorders as well as their emerging roles in senescence [14, 15]. The relative expression of the lncRNA/mRNA signatures were analyzed by preparing reaction mixer with FastStart Universal SYBR Green Master (Roche) and the corresponding gene-specific primers (Sigma) with diluted cDNA and final volume made up to 20 μL using nuclease-free water. Quantification and analysis were carried out in LightCycler® 96 real-time PCR System (Roche). The target gene expression was normalized to the house-keeping gene 18SrRNA (lncRNA) and β-actin (mRNA), and relative expression was determined using $2^{-\Delta\Delta CT}$ method. Non-template control (NTC) was also performed for each reaction assay plate.

DNA isolation and measurement of telomere length

For the measurement of telomere length, DNA was isolated from the whole blood by phenol–chloroform extraction and ethanol precipitation [16]. Relative telomere length was determined by real-time PCR approach as previously described by Cawthon [17] with a minor modification in the PCR temperature conditions. This method measures the factor by which the ratio of telomere repeat copy number to single-gene copy number differs between a sample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers (S), which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) was represented as T/S ratio—a reflection of relative telomere length [3].

Statistical analysis

All data are represented as mean ± standard error mean (SEM) unless otherwise mentioned as standard deviation (SD). Based on our pilot study on the expression levels of lncRNAs and using the SPSS software, the minimum sample size required for the study was calculated as 28 in each group considering the level of significance set at 0.05 and the statistical power at 0.90. Comparison between groups was performed using the independent sample Student *t* test with $p < 0.05$ as the criterion for statistical significance. Pearson correlation analysis was done between variables and the risk factors. Binary logistic regression analysis was performed to show the association between lncRNAs (independent variable) and diabetes (dependent variable). All analyses were done using SPSS Statistics (version 20.0) and GraphPad Prism (version 6).

Results

Clinical and biochemical characteristics of the study groups

Clinical and biochemical characteristics of the study subjects are depicted in Table 1. BMI and waist circumference were slightly and significantly higher in patients with type 2 diabetes compared to control subjects. Patients with type 2 diabetes exhibited significantly ($p < 0.001$) increased fasting plasma glucose and HbA1c compared to the control subjects. T2DM patients were also hyperinsulinemic and insulin resistant as characterized by significantly elevated fasting insulin levels and HOMA-IR values, respectively. Blood pressure and lipid parameters did not differ significantly between the groups.

Altered lncRNA signatures in T2DM

Compared to control subjects, expression profiling of lncRNAs in PBMCs from type 2 diabetes patients showed significantly ($p < 0.05$) increased levels of HOTAIR, MEG3, LET, MALAT1, MIAT, CDKN2BAS1/ANRIL, XIST, PANDA, GAS5, Linc-p21, ENST00000550337.1, PLUTO, and NBR2 (Fig. 1). In contrast, lncRNA expression patterns of THRIL and SALRNA1 were significantly ($p < 0.05$) decreased in patients with T2DM compared to control subjects (Fig. 1).

Table 1 Clinical and biochemical characterization of the study subjects

Parameter	Normal glucose tolerance [NGT] ($n = 32$)	Type 2 diabetes mellitus [T2DM] ($n = 30$)	p value
Age (years)	44 ± 8	46 ± 8	0.218
Gender—male (female)	18 (14)	18 (12)	–
Body mass index (kg/m^2)	25 ± 3.1	27 ± 4	*0.015*
Waist circumference (cm)	85 ± 8	94 ± 9	*< 0.001*
Fasting plasma glucose (mg/dL)	87 ± 9	136 ± 24	*< 0.001*
Glycated hemoglobin—HbA1c (%)	5.6 ± 0.34	8.1 ± 1.9	*< 0.001*
HOMA-IR	1.8 ± 0.8	6.9 ± 3	*< 0.001*
Fasting insulin (μIU/mL)	8.6 ± 3.5	22 ± 7.2	*< 0.001*
Systolic blood pressure (mmHg)	120 ± 25	131 ± 21	0.079
Diastolic blood pressure (mmHg)	79 ± 13	80 ± 8	0.795
Total cholesterol (mg/dL)	174 ± 28	169 ± 37	0.545
Serum triglycerides (mg/dL)	132 ± 71	138 ± 49	0.737
HDL cholesterol (mg/dL)	41 ± 10	39 ± 7	0.352
LDL cholesterol (mg/dL)	107 ± 21	102 ± 34	0.568
VLDL	27 ± 14	28 ± 10	0.732

Data represented as mean ± SD. Italicized value represents statistically significant compared to NGT

Augmentation of HDAC3 and impaired Sirt1 expression in T2DM

Transcriptional profiling revealed that mRNA expression of HDAC3 was significantly ($p < 0.05$) increased while the Sirt1 level was significantly ($p < 0.05$) decreased in patients with type 2 diabetes compared to control subjects (Fig. 2).

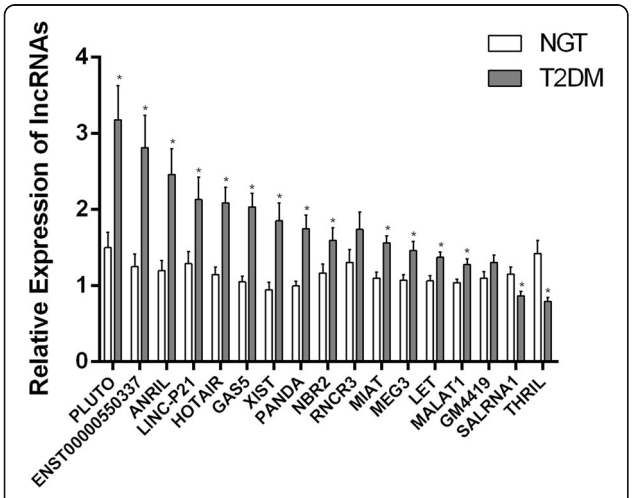

Fig. 1 Quantitative real-time PCR analysis of a panel of lncRNA expression levels in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

Altered senescence, inflammation, and telomere length in T2DM

At the transcriptional level, senescence markers, viz., p53, p21, p16, and β-galactosidase 1 (GLB1), were significantly ($p < 0.05$) elevated in patients with type 2 diabetes compared to control subjects (Fig. 3a). As a final read-out of augmented cellular senescence, patients with T2DM were also characterized by significantly ($p < 0.05$) shortened telomeres compared to control subjects (Fig. 3b). Interestingly, mRNA expression levels of proinflammatory gene mediators, viz. TNF-α, IL6, MCP1 and IL1-β, were also significantly upregulated ($p < 0.05$) in PBMCs from patients with type 2 diabetes, implying an acquisition state of senescence-associated secretory phenotype (Fig. 4).

Correlation analysis

A detailed correlation analysis of lncRNAs with various clinical and biochemical parameters (Additional file 1: Table S1) and molecular parameters (Additional file 2: Table S2) of the study subjects were summarized in the supplement tables. Majority of the altered lncRNAs were positively correlated with poor glycemic control, insulin resistance, transcriptional markers of senescence, inflammation, and HDAC3 and negatively correlated with telomere length. In contrast, expression levels of lncRNAs, viz., SALRNA1 and THRIL, were negatively correlated to glycemic control, insulin resistance, markers of senescence, inflammation, and HDAC3 and positively correlated to telomere length.

Logistic regression analysis

Logistic regression analysis using type 2 diabetes as dependent variable revealed that altered expression levels of lncRNAs, viz., PLUTO, ENST00000550337.1, CDKN2BAS1, LincRNA-P21, HOTAIR, GAS5, XIST, PANDA, NBR2, MIAT, MEG3, LET, MALAT1, SALRNA1, and THRIL, were associated significantly with T2DM, and this statistical significance was persisted even after adjusting for confounding factors like age and BMI. Interestingly, this statistical association was lost when adjusted for HOMA-IR and senescence markers. This implies that the association between lncRNAs and T2DM could be closely linked to insulin resistance and accelerated senescence with downstream inflammatory signaling (Table 2).

Discussion

Recent literature implies that the dysregulation of lncRNA expression and functionality contributes to several pathophysiological states as several lncRNAs get

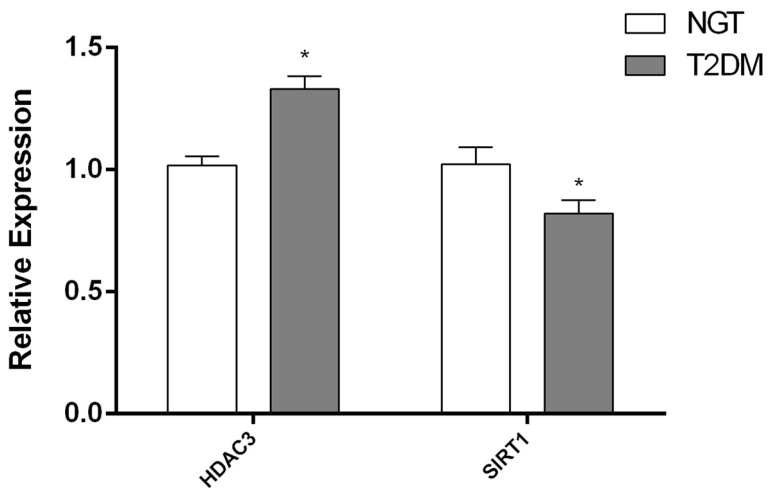

Fig. 2 Quantitative real-time PCR analysis of HDAC3 and SIRT1 in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

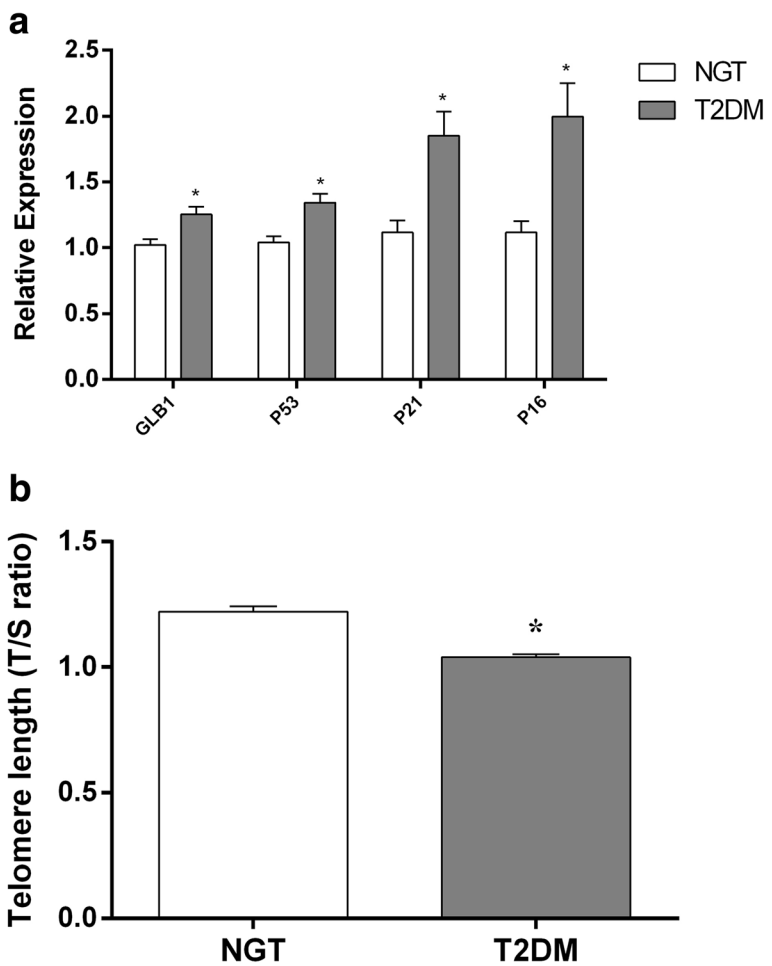

Fig. 3 Quantitative real-time PCR analysis of senescence marker gene expression levels, viz., GLB1, P53, P21, and P16 (**a**), and telomere length (**b**) in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

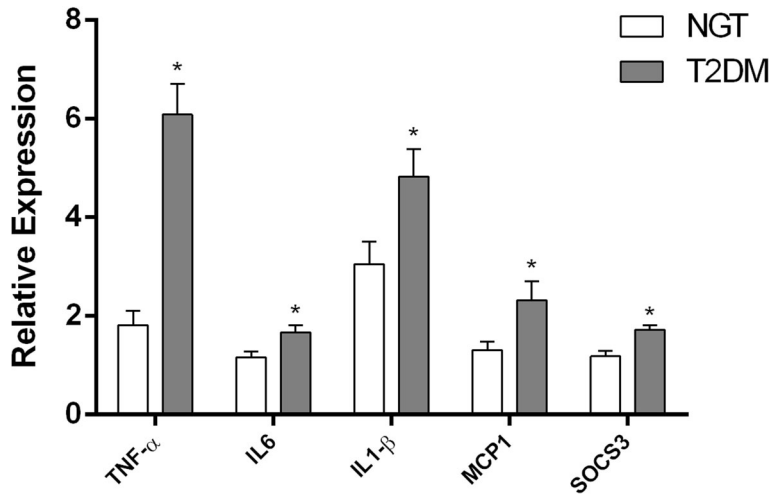

Fig. 4 Quantitative real-time PCR analysis of inflammatory signature gene expression levels, viz., TNFα, IL6, MCP1, IL1β, and SOCS3 in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

validated as bona fide prognostic/diagnostic markers and drug targets [9, 18, 19]. The role of lncRNAs in the pathogenesis of type 2 diabetes mellitus and related complications has only recently been recognized, but there is already some evidence for their involvement in many of the pathophysiological mechanisms that are linked to the genesis and progression of disease [20, 21]. Despite the fact that Asian Indians are highly insulin resistant [10], more prone to develop type 2 diabetes mellitus (T2DM) and associated vascular complications [11], and exhibit increased susceptibility to early β-cell dysfunction [22], there is virtually lack of data on the role of lncRNAs in the clinical diabetes setting. Our study is the first report from India to show an association of altered signatures of lncRNAs in T2DM with pathological connectivity reflected by poor glycemic control, insulin resistance, accelerated cellular senescence, and meta-inflammation.

Table 2 Binary logistic regression analysis using type 2 diabetes as dependent variable

	Unadjusted		Adjusted for age and BMI		Adjusted for HOMA-IR		Adjusted for senescence markers (GLB, P53, P21, P16, and TL)		Adjusted for inflammatory markers (TNF-α, IL6, MCP1, IL1-β, and SOCS3)	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
PLUTO	1.721	*0.005*	1.827	*0.003*	23.673	0.063	1.848	0.210	2.204	*0.025*
ENST00000550337.1	2.023	*0.004*	1.925	*0.013*	2.984	0.132	33.73	0.184	4.026	*0.038*
CDKN2BAS1	3.173	*0.006*	4.925	*0.002*	4.188	0.068	2.741	0.100	2.995	*0.044*
lincRNA-p21	1.867	*0.021*	1.970	*0.033*	3.492	0.096	2.283	0.311	6.395	*0.013*
HOTAIR	4.348	*0.001*	5.256	*0.001*	2.556	0.342	1.651	0.524	8.125	*0.015*
GAS5	10.642	*0.001*	14.054	*0.001*	20.820	0.226	22.512	0.128	12.214	0.069
XIST	0.388	*0.003*	3.824	*0.004*	3.677	0.145	2.318	0.386	3.166	*0.024*
PANDA	7.960	*0.003*	15.737	*0.002*	30.052	*0.041*	27.726	0.151	3.548	0.253
NBR2	2.045	*0.041*	1.728	0.159	1.496	0.522	1.869	0.443	2.675	0.141
RNCR3	1.464	0.144	1.650	0.065	1.795	0.394	1.582	0.410	1.740	0.225
MIAT	6.591	*0.002*	5.293	*0.012*	8.383	0.235	8.753	0.181	5.388	0.118
MEG3	5.669	*0.013*	6.444	*0.017*	17.060	0.141	12.830	0.063	57.903	*0.023*
LET	7.116	*0.014*	5.806	*0.036*	6.736	0.068	4.399	0.534	2.079	0.584
MALAT1	9.945	*0.008*	5.156	*0.046*	4.712	0.193	12.858	0.343	33.033	0.086
GM4419	2.468	0.104	2.142	0.242	3.254	0.232	4.832	0.293	1.937	0.433
SALRNA1	0.161	*0.013*	0.114	*0.009*	0.029	0.127	0.072	0.057	0.092	0.100
THRIL	0.047	*0.001*	0.529	*0.001*	0.013	0.084	0.063	0.139	0.333	0.271

Our study is in consistent with the recent literature of several lncRNAs upregulated in diabetes state. In support of our findings, increased expression of GAS5 [23] and lncRNA ENST00000550337.1 [24] was reported in type 2 diabetes even with high diagnostic claim and biomarker value. A role for lncRNAs XIST [25] and GM4419 [26] was implicated in diabetic nephropathy while alterations in PANDA [27] and NBR2 [28] P21 [29] were linked to cellular senescence, AMPK regulation, and liver fibrosis, respectively. Expression levels of lncRNA-LET was shown to be decreased in a certain type of cancers [30], but we observed it to be upregulated in patients with type 2 diabetes. MIAT is identified to be involved in various diseases, particularly myocardial infarction, diabetic retinopathy, and various other microvascular complications [31]. Similarly, lncRNA RNCR3 was shown to be increased in retinal vasculature of an animal model as well as in vitro cell model [32]. While lncRNA PLUTO has been shown to be downregulated in islets from donors who are patients with type 2 diabetes and pre-diabetes subjects [33], our study observed a highly significant upregulation of PLUTO in patients with type 2 diabetes. Previous studies also reported that upregulated expression of lncRNA MALAT1 was linked to hyperglycemia-induced inflammation and endothelial dysfunction [34], diabetic nephropathy [35], and gestational diabetes mellitus [36]. In vitro studies demonstrated that HOTAIR interacts with the various chromatin-modifying enzymes and thereby participates in the regulation of gene expression [37]. A functional role for HOTAIR in the diabetes pathogenesis is yet to be established; however, its role has been hinted to be associated with regional adiposity [38]. lncRNA MEG3 has an important regulatory role in beta cell function [39], and the knock-down of MEG3 has been shown contributing to the pathology of diabetic microvascular complication [40]. In contrast, MEG3 gene expression was shown upregulated in the hepatocytes from mice fed with high-fat diet as well as in ob/ob mice and this has been linked to increased hepatic gluconeogenesis [41].

Our study provides the first preliminary evidence that expression of the long non-coding RNAs, THRIL, and SALRNA1 were decreased in patients with type 2 diabetes and negatively correlated with hyperglycemia, senescence, and inflammation. THRIL was shown to regulate TNF-α expression through an epigenetic mechanism, and TNF-α can also reduce THRIL expression via a negative feedback action [42]. Similarly, SAL-RNA1 was earlier identified as putative age-delaying lncRNA, since its reduction with small inhibitory RNAs (siRNA) induced rapid aging changes of the fibroblasts, such as large cell morphology, positive β-galactosidase activity, and upregulation of p53 [43]. Notably, lncRNA ANRIL shown upregulated in our study was also linked to CDKN2A/B, a strong type 2 diabetes risk gene variant [44, 45].

It is interesting to note that the majority of differentially expressed lncRNAs in patients with type 2 diabetes observed in our study are involved in cell cycle regulation and senescence and their expression levels correlated to poor glycemic control, insulin resistance, accelerated senescence, and inflammation. Several lncRNAs were reported to influence the molecular processes that underlie age-associated phenotypes and play an important role in accelerated aging [4, 46]. Type 2 diabetes has been linked to cellular senescence, senescence-associated secretory phenotype (SASP), and accelerated aging [47, 48], and our lab was the first one in the world literature to report an association of increased telomere shortening in patients with type 2 diabetes [2, 3]. Earlier, we have also shown increased HDAC3 epigenetic signature in patients with type 2 diabetes [49], and in the present study, there was a positive correlation of HDAC3 mRNA expression with majority of the lncRNAs and this endorses the concerted and coordinated interactions between lncRNAs and histone modifications [50].

Our work offers an avenue for several translational applications including a role of lncRNAs in lifestyle changes. Recent findings suggest a putative role of non-coding RNAs in physical activity and several miRNAs have been identified as modulators of exercise-induced adaption at both systemic and tissue levels [51]. Contrast to miRNAs, little is known about the role of long non-coding RNAs (lncRNAs) in exercise. Identification of the role of lncRNAs in exercise will improve our understanding of exercise physiology and has the potential to enhance the application of current therapeutic approaches. In fact, a micropeptide encoded by a putative lncRNA has been shown to regulate muscle performance [52]. Although very little is known about the relationship between lncRNAs and dietary factors, it appears that dietary manipulation could also beneficially alter the expression of lncRNAs and thereby ensure health [53].

One of the limitations of our study is of its cross-sectional nature as well as small sample size, and hence, the findings of the study and its conclusions should be interpreted with caution. From this pilot study, we could not extrapolate causal link of alterations in lncRNAs with type 2 diabetes, and it needs replication and prospective follow-up studies. Secondly, considering the tissue-specific and heterogeneous actions of lncRNAs, the alterations seen in PBMCs might only mirror disease-pathology directionality. However, the altered expression profile of lncRNAs in PBMCs has been shown to reflect the pathophysiology in different disease states including multiple sclerosis [54], myocardial infarction [55], and rheumatoid arthritis [56]. In fact, a recent study of deep RNA sequencing uncovered a repertoire

of human macrophage lncRNAs modulated by macrophage activation and closely linked it to the pathophysiology of cardiometabolic diseases [57].

Conclusion

To conclude, our study is of its first kind in India to report altered lncRNA profiles linked to poor glycemic control, insulin resistance, senescence, and proinflammation in patients with type 2 diabetes. A better understanding of the mechanisms underlying the functions of lncRNAs will help us to understand the ever-expanding pathophysiology of diabetes and its complications and thereby adapt to prevention strategies as well as to develop novel therapeutic agents.

Additional files

Additional file 1: Table S1. Correlation analysis of LncRNAs with clinical and biochemical parameters. (DOCX 21 kb)

Additional file 2: Table S2. Correlation analysis of LncRNAs with molecular parameters. (DOCX 18 kb)

Abbreviations

BMI: Body mass index; GLB1: β-Galactosidase 1; HbA1C: Glycated hemoglobin; HDAC: Histone deacetylase; HOMA-IR: Homeostatic model assessment-insulin resistance; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; lncRNAs: Long non-coding RNAs; MCP-1: Monocyte chemoattractant protein 1; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NGT: Normal glucose tolerance; P16: Cyclin-dependent kinase inhibitor 2A; P21: Cyclin-dependent kinase inhibitor 1A; P53: Tumor protein/tumor suppressor 53; PBMCs: Peripheral blood mononuclear cells; SASP: Senescence-associated secretory phenotype; Sirt1: Sirtuin (silent mating-type information regulation 2 homolog) 1; SOCS-3: Suppressor of cytokine signaling 3; T2DM: Type 2 diabetes mellitus; TNF-α: Tumor necrosis factor-α

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Availability of data and materials

The authors consent to the availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MB conceived, designed, supervised, and commented on all drafts of this paper. CS and PP coordinated the clinical sample collection, conducted the overall experiments, and participated in the data collection and analysis and molecular investigations and helped in the drafts. VM and MB contributed to the data interpretation and manuscript completion. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee of the Madras Diabetes Research Foundation and conducted according to the principles of Declaration of Helsinki. Written informed consent was obtained from all the study participants prior to the start of the study.

Consent for publication

Institutional consent form is inclusive of data protection and consent for research publication.
All the authors approved the manuscript and consented for publication.

Competing interests

The authors declare that they have no competing interests.

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Research highlight

Fast forward aging link in diabetes

Researchers have shown a molecular connection between the nuclear and mitochondrial aging processes that occur in patients with type 2 diabetes[1].

The human body has a chronological age as also a biological age. The biological age is represented by the length of telomere — the DNA sequence at the end of each chromosome, like the plastic tips on shoelaces. The telomeres get shorter each time a cell divides. Short telomeres reflect accelerated ageing.

Many recently discovered genes that can be manipulated to slow the aging process also belong to pathways involved in the control of metabolism. Metabolic syndrome, in addition to being a precursor of metabolic disorders such as type 2 diabetes mellitus (T2DM) and cardiovascular disease, has been shown to be a sign of premature aging. Diabetes is a state of accelerated aging.

While telomere shortening is associated with T2DM, there is a lack of studies that explore the relationship among all the biomarkers — telomere length, oxidative stress, mitochondrial DNA (mtDNA) content, and the levels of adiponectin (a protein produced by fat cells that may play an important role in the development of obesity).

The researchers reasoned that the susceptibility to develop T2DM and cardiovascular diseases in Asian Indians could be explained by studying all these emerging biomarkers. "In a clinical setting, we have shown the existence of a molecular connection between the nuclear and mitochondrial ageing processes which occur in patients with type 2 diabetes," says lead author Muthuswamy Balasubramanyam.

Unlike chronological aging, accelerated aging can be reversed. "In other words, maintenance of appropriate mitochondrial function and telomere length either by pharmacological means or lifestyle modification will have promising therapeutic potential for Type 2 diabetes and associated vascular disorders", he adds.
The authors of this work are from: Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, Chennai, India.

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Research highlight

Aging fat cells signal diabetes

Researchers have found that aging fat cells could signal diabetes. In a new research, they show that aging fat cells provide a molecular link to a person's insulin resistance, a precursor for many metabolic diseases including diabetes[1].

The researchers have shown in animal models that it is possible to selectively target aging cells, eliminate them and delay or prevent age-related pathologies. This calls for further research on 'clearing away old cells' either pharmacologically or by lifestyle modifications to achieve healthy aging.

In response to a variety of stress signals, including nutrient deprivation, oxidative stress, dysfunctional telomeres and DNA damage, normally dividing cells can permanently withdraw from the typical cell cycle. These cells are then said to be in a state of 'cellular senescence', where their capacity to replicate is destroyed. Growing evidence suggests that these senescent cells contribute to aging in a variety of organisms, including mice and humans.

Senescent cells lurk in our tissues. They escape elimination due to impaired programmed cell death or altered immune surveillance. Senescent cells have been reported to behave badly, secrete chemicals that degrade surrounding tissue and harm neighboring cells. Their increasing omnipresence contributes to accelerated aging and age-related pathologies.

"In our study, fat cells subjected to oxidative stress became senescent associated secretory phenotype (SASP), started signalling proinflammation, exhibited shortened telomeres and became insulin-resistant. This is a hallmark characteristic of diabetes" says lead author Muthuswamy Balasubramanyam.

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Research highlight

Why lean diabetics could be metabolically obese

Researchers have found molecular evidence to answer why some type 2 diabetics who appear lean are actually 'metabolically obese'[1].

Overweight and obesity are known to be important risk factors for diabetes and cardiovascular diseases. In Asia, obesity rates do not directly correspond with diabetes rates. India has a low prevalence of obesity but notably high rates of type 2 diabetes.

In Asian populations, a higher risk of diabetes starts at a lower body mass index than in Europeans. Thus, one of the factors that contributes to the diabetes epidemic in Asians is the 'normal-weight metabolically obese' phenotype. Till now, the biochemical or molecular basis for 'metabolic obesity' was poorly understood.

To probe this, the researchers measured the size of fat cells (adipocytes) from human visceral and subcutaneous fats and found that lean diabetics had higher adipocyte cell size (adipocyte hypertrophy) than control subjects. Also, adipose tissue from these lean diabetics showed faster aging (senescence) as evident from their shortened telomeres. They also had a secretory profile going by the low levels of adiponectin, the protein involved in regulating glucose levels as well as fatty acid breakdown.

"When such fat cells switch to a senescent and proinflammatory phenotype, they also change their job from 'fat storage' to 'fat spillage'. This fat accumulation complicates functioning of several other organs including the heart and triggers cardiovascular disease in diabetes patients" says lead researcher Muthuswamy Balasubramanyam. The study exposes adipocyte hypertrophy and senescence as targets for new drug discoveries, he adds.

The research urges timely prevention and management to reduce adverse outcomes in all patients with type 2 diabetes, particularly in metabolically obese normal-weight patients, who may have a false sense of protection because they are not overweight or obese.

The authors of this work are from: *Madras Diabetes Research Foundation, Chennai, Dr. Mohan's Diabetes Specialities Centre, Chennai & LifeLine Multi-Speciality Hospitals, Chennai, India.*

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