Ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)

1. Pinna NK, Anjana RM, Saxena S, Dutta A, Gnanaprakash V, Rameshkumar G, Aswath S, Raghavan S, Shanthirani CS, Radha V, **Balasubramanyam, M**. et al. Trans-ethnic gut microbial signatures of prediabetic subjects from India and Denmark. Genome Med. 2021;13(1):36. doi: 10.1186/s13073-021-00851-9.

   Recent studies have indicated an association of gut microbiota and microbial metabolites with type 2 diabetes mellitus (T2D). However, there is lack of studies on the large-scale investigation of the gut microbiota of "prediabetic" individuals. Uniquely this bilateral study has reported the association of distinct trans-ethnic gut microbiome and inflammation signatures with prediabetes in Indian and Danish populations. While the overall results confirm a state of proinflammation as early as in prediabetes, the Indian cohort exhibited a characteristic pattern of abundance of inflammatory markers indicating low-grade intestinal inflammation at an overall population level, irrespective of glycemic status.

2. Prabu P, Poongothai S, Shanthirani CS, Anjana RM, Mohan V, **Balasubramanyam M**. Altered circulatory levels of miR-128, BDNF, cortisol and shortened telomeres in patients with type 2 diabetes and depression. Acta Diabetol. 2020, 57(7):799-807

   In this study, patients with type 2 diabetes and depression were shown to exhibit increased circulatory levels of miR-128 and serum cortisol and decreased levels of BDNF and shortened telomeres. These neuroendocrine signatures were more markedly altered in those with combined diabetes and depression.

3. Soundararajan A, Prabu P, Mohan V, Gibert Y, **Balasubramanyam M**. Novel insights of elevated systemic levels of bisphenol-A (BPA) linked to poor glycemic control, accelerated cellular senescence and insulin resistance in patients with type 2 diabetes. Mol Cell Biochem. 2019; 458(1-2):171-183

   Increased systemic levels of bisphenol-A (BPA) linked to the etiology and pathogenesis of type 2 diabetes: While there is cause of concern on endocrine disruptors (like BPA) as environmental risk for type 2 diabetes and other metabolic disorders, there is lack of data on this in India. Our work not only demonstrated elevated systemic levels of BPA in patients with type 2 diabetes but also delineated its association with cellular alterations of several signatures including accelerated senescence.

4. Prabu P, Rome S, Sathishkumar C, Gastebois C, Meugnier E, Mohan V, **Balasubramanyam M**. MicroRNAs from urinary extracellular vesicles are non-invasive early biomarkers of diabetic nephropathy in type 2 diabetes patients with the 'Asian Indian phenotype'. Diabetes Metab. 2019; 45(3):276-285

As microRNAs from extracellular vesicles (EVs) have been proposed as promising biomarkers for a number of diseases, one of our studies explored their potential as urine-based biomarkers of diabetic nephropathy (DN) in a discovery and validation cohort. A panel of four urinary EV miRNA signatures was found to identify patients with microalbuminuria (MIC). With the limitations of MIC test (a gold-standard for predicting DN), this study endorses the clinical utility of urinary miRNAs from EVs as non-invasive 'liquid biopsies' to stratify patients at risk of developing macroalbuminuria (MAC) and diabetic kidney disease. This study has been quoted in the Asia Research News as '**Liquid-biopsy microRNA markers to predict risk for diabetic kidney disease'.**

5. Sathishkumar C, Prabu P, Mohan V**, Balasubramanyam M**. Linking a role of lncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes. Hum Genomics. 2018 Aug 23;12(1):41. doi: 10.1186/s40246-018-0173-3.

   Linking a role of lncRNAs (long non-coding RNAs) in the etiology of Type 2 diabetes: This work unraveled a new-biology link in the etiology of type 2 diabetes as we demonstrated an association of altered long non-coding RNAs (LncRNAs) with accelerated senescence, inflammation and insulin resistance in patients with type diabetes. This got wide science media attention as appeared in European Medical Journal site as: **'Altered LncRNAs signify an upstream link in the etiology of type 2 diabetes'.** Highlights of this study include the following: 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.

6. Prabu P, Rome S, Sathishkumar C, Aravind S, Mahalingam B, Shanthirani CS, Gastebois C, Villard A, Mohan V, **Balasubramanyam M.** Circulating MiRNAs of 'Asian Indian Phenotype' Identified in Subjects with Impaired Glucose Tolerance and Patients with Type 2 Diabetes. PLoS One. 2015 May 28;10(5):e0128372. doi: 10.1371/journal.pone.0128372. eCollection 2015.

   Circulatory microRNAs characteristic of 'Asian Indian phenotype": This is the first study in India to expose the biomarker role of circulatory microRNAs characteristic of 'Asian Indian phenotype' in type 2 diabetes patients. As the technologies related to miRNA measurement are evolving, our study implied that specific miRNA profile either singly or as a panel would qualify as potential tool for personalized medicine. This study has been highly appreciated in the media and quoted in ResearchSEA as **'MicroRNA markers for Madhumeha'.**

7. Monickaraj, F., Gokulakrishnan, K., Prabu, P., Sathishkumar, C., Anjana, R.M., Rajkumar, J.S., Mohan, V & **Balasubramanyam, M**. Convergence of adipocyte hypertrophy, telomere shortening and hypoadiponectinemia in obese subjects and in patients with Type 2 diabetes. Clinical Biochemistry 2012, 45(16-17):1432-8

   One of the factors that contribute to accelerated diabetes epidemic in Asians is referred to as "normal-weight metabolically obese" phenotype. However, the biochemical or molecular basis for **'metabolic obesity'** is poorly understood. In this study, the measurements of adipocyte cell size from human visceral and subcutaneous fat tissue demonstrated that non-obese diabetics are characterized by increased adipocyte cell size (adipocyte hypertrophy). In addition, adipose tissue from these lean diabetics showed increased senescence as evident from shortened telomeres and compromised secretory profile as evident from low levels of circulatory adiponectin. This is again for the first-time in India, the telomere length has been reported utilizing adipose tissue. The finding by Balasubramanyam and co-workers assumed greater significance because it is in a clinical setting, they unraveled the molecular basis for 'metabolic obesity' in diabetic patients. This study exposed adipocyte hypertrophy and senescence pathway as targets for new drug discoveries. This study has been quoted in Nature India as **'Why lean diabetics could be metabolically obese'.**

8. Monickaraj, F., Aravind, S., Gokulakrishnan, K., Satishkumar, C., Prabu, P., Prabu, D., Viswanathan Mohan, V. & **M. Balasubramanyam**. Accelerated ageing as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with Type 2 diabetes. Molecular and Cellular Biochemistry 2012, 365(1-2):343-50

   This study by Balasubramanyam and colleagues unraveled clustering of biomarkers of accelerated aging in patients with type 2 diabetes as evidenced by an association of shortened telomeres with decreased mtDNA, hypoadiponectinemia and increased oxidative stress. The finding assumes greater significance because, it is in a clinical setting, we showed the existence of a molecular connection between the nuclear and mitochondrial ageing processes which occur in a 'fast-forward' way in patients with type 2 diabetes. Unlike the chronological aging, accelerated aging could be reversed and hence amenable for intervention. It is emphasized that 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. This study has been quoted in Nature India as **'Fast forward aging link in diabetes'**.

9. **Balasubramanyam M**, Aravind S, Gokulakrishnan K, Prabu P, Sathishkumar C, Ranjani H and Mohan, V. Impaired miR-146a expression links subclinical inflammation and insulin resistance in type 2 diabetes. Mol Cell Biochem. 2011 351(1-2):197-205

   Over the past 5 years, it has become increasingly clear that miRNAs are not only important for normal organismal development and physiology, but also in the pathologies related to many metabolic diseases. While Indians are considered highly insulin-resistant, prone to develop diabetes and vascular diseases and subclinical inflammation is a part of the pathogenic mechanisms, the regulatory mechanisms of pro-inflammation are poorly

understood. Utilizing peripheral blood mononuclear cells (PBMCs) as a surrogate cell model, Balasubramanyam and colleagues demonstrated impaired miR-146a expression linking subclinical inflammation and insulin resistance in type 2 diabetes. In this study, miR-146a exhibited a negative association with several proinflammatory target genes such as TRAF-6 and NFkB and circulatory levels of TNFα and IL-6. Interestingly, miR-146a levels also showed a negative association with insulin resistance and glycated hemoglobin. While the work demonstrated an association of impaired miR-146a with subclinical inflammation in patients with type 2 diabetes, it also provided an avenue to look for specific and appropriate miRNA mimics including small molecule modulators as novel anti-inflammatory therapeutic measures. **This study has been quoted in Nature India as 'RNA clue to inflammation'.**

10. Adaikalakoteswari A, **Balasubramanyam M** and Mohan V. Telomere shortening occurs in patients with Type 2 diabetes. Diabetic Medicine, 22, 1151–1156, 2005.

    This landmark work is the first study in the world literature to demonstrate shortened telomeres in patients with type 2 diabetes. Seeking a dynamic and long-term biomarker of molecular stress signaling in the genesis of diabetes and its complications, Balasubramanyam and colleagues reported shortened telomeres in Asian Indian patients with type 2 diabetes. Several of the continuing works on this direction demonstrated **accelerated aging** as an underlying cause for insulin resistance and type 2 diabetes which could be reversible and hence amenable for therapeutic and/or lifestyle intervention.

DOI: 10.1111/j.1464-5491.2005.01574.x

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

Accepted 27 October 2004

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.

Diabet. Med. 22, 1151–1156 (2005)

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

Sathishkumar et al. Human Genomics (2018) 12:41
https://doi.org/10.1186/s40246-018-0173-3

Human Genomics

PRIMARY RESEARCH Open Access

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

Acta Diabetologica
https://doi.org/10.1007/s00592-020-01486-9

ORIGINAL ARTICLE

Altered circulatory levels of miR-128, BDNF, cortisol and shortened telomeres in patients with type 2 diabetes and depression

Paramasivam Prabu[1] · Subramani Poongothai[1] · Coimbatore Subramanian Shanthirani[1] · Ranjit Mohan Anjana[1] · Viswanathan Mohan[1] · Muthuswamy Balasubramanyam[1]

Received: 18 November 2019 / Accepted: 20 January 2020

Abstract

Aims Several studies have reported the role of biomarkers either in diabetes or depression. The present study is aimed at profiling the circulating levels of miR-128, brain-derived neurotrophic factor (BDNF), cortisol and telomere length in patients with type 2 diabetes with and without depression compared to individuals with normal glucose tolerance.

Methods Study subjects ($n = 160$) were recruited from an ongoing epidemiological study in southern India. Non-diabetic and diabetic individuals were diagnosed as per the World Health Organization criteria. Depression score was derived using PHQ-12 questionnaire. Real-time quantitative PCR and ELISA methodologies were used to quantify the biomarkers.

Results Circulatory levels of miR-128 and cortisol were significantly ($p < 0.05$) increased with decreased BDNF levels and shortened telomeres in T2DM patients with or without depression compared to NGT individuals. T2DM patients with depression had the highest levels of miR-128 and cortisol and lowest levels of BDNF and telomere length compared to other groups. Pearson correlation analysis showed miR-128 levels were negatively associated with BDNF, telomere length and HDL cholesterol and positively correlated with cortisol, depression score, poor glycemic control and insulin resistance. Regression analysis confirmed that miR-128 was significantly associated with depression score even after adjusted for several confounding factors. However, this association was lost when adjusted for cortisol or telomere length.

Conclusions Patients with type 2 diabetes and depression exhibited increased circulatory levels of miR-128 and serum cortisol and decreased levels of BDNF and shortened telomeres. These neuroendocrine signatures were more markedly altered in those with combined diabetes and depression.

Keywords Diabetes · Depression · MicroRNA · Telomere shortening · BDNF · Cortisol

Introduction

With the ever-increasing prevalence of type 2 diabetes mellitus (T2DM) worldwide and particularly in the developing countries like India, the societal and economic burden of diabetes is significant [1]. Indeed, the impact of T2DM on quality of life, life expectancy, and premature morbidity and mortality is substantial. While premature deaths among patients with T2DM are primarily attributable to macro- and microvascular complications of this disease, living with T2DM and its comorbidities can also have important mental health consequences that can affect the therapeutic response and treatment of the disorder [2]. It is also known that depression is a major comorbid disorder in individuals with T2DM [3]. Depression is a well-known risk factor for noncompliance with medical treatment [4], and this can be particularly problematic for a chronic life-long condition

Managed by Massimo Porta.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00592-020-01486-9) contains supplementary material, which is available to authorized users.

✉ Muthuswamy Balasubramanyam
baluglobaldiab@gmail.com
http://www.mdrf.in

[1] Department of Cell and Molecular Biology, Madras Diabetes Research Foundation (MDRF) and Dr. Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control & ICMR Centre for Advanced Research on Diabetes, No 4, Conran Smith Road, Gopalapuram, Chennai 600086, India

Pinna et al. Genome Medicine (2021) 13:36
https://doi.org/10.1186/s13073-021-00851-9

Genome Medicine

RESEARCH Open Access

Trans-ethnic gut microbial signatures of prediabetic subjects from India and Denmark

Nishal Kumar Pinna[1†], Ranjit Mohan Anjana[2†], Shruti Saxena[3†], Anirban Dutta[1†], Visvanathan Gnanaprakash[2], Gnanavadivel Rameshkumar[2], Sukumaran Aswath[2], Srividhya Raghavan[2], Coimbatore Subramanian Shanthi Rani[2], Venkatesan Radha[2], Muthuswamy Balasubramanyam[2,4], Archana Pant[3], Trine Nielsen[5], Torben Jørgensen[6], Kristine Færch[7], Alireza Kashani[5,8], Maria Camila Alvarez Silva[5], Henrik Vestergaard[5], Tue Haldor Hansen[5,9], Torben Hansen[5], Manimozhiyan Arumugam[5], Gopinath Balakrish Nair[3], Bhabatosh Das[3*], Oluf Pedersen[5*], Viswanathan Mohan[2*] and Sharmila Shekhar Mande[1*]

Abstract

Background: Recent studies have indicated an association of gut microbiota and microbial metabolites with type 2 diabetes mellitus (T2D). However, large-scale investigation of the gut microbiota of "prediabetic" (PD) subjects has not been reported. Identifying robust gut microbiome signatures of prediabetes and characterizing early prediabetic stages is important for the understanding of disease development and could be crucial in early diagnosis and prevention.

Methods: The current study performed amplification and sequencing on the variable regions (V1–V5) of the 16S rRNA genes to profile and compare gut microbiota of prediabetic individuals ($N = 262$) with normoglycemic individuals ($N = 275$) from two cohorts in India and Denmark. Similarly, fasting serum inflammatory biomarkers were profiled from the study participants.

(Continued on next page)

* Correspondence: bhabatosh@thsti.res.in; oluf@sund.ku.dk; drmohans@diabetes.ind.in; sharmila.mande@tcs.com
†Nishal Kumar Pinna, Ranjit Mohan Anjana, Shruti Saxena and Anirban Dutta contributed equally to this work.
[3]Molecular Genetics Laboratory, Infections and Immunology, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, 3rd Milestone, Faridabad – Gurgaon Expressway, PO box #04, Faridabad 121001, India
[5]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Blegdamsvej 3B, Maersk Tower, Building: 07-8-55, DK-2200 Copenhagen N, Denmark
[2]Madras Diabetes Research Foundation, No. 4, Conran Smith Road, Gopalapuram, Chennai 600 086, India
[1]TCS Research, Tata Consultancy Services Limited, 54B Hadapsar Industrial Estate, Pune 411013, India
Full list of author information is available at the end of the article

Diabetes & Metabolism xxx (2018) xxx–xxx

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

MicroRNAs from urinary extracellular vesicles are non-invasive early biomarkers of diabetic nephropathy in type 2 diabetes patients with the 'Asian Indian phenotype'

P. Prabu [a], S. Rome [b], C. Sathishkumar [a], C. Gastebois [b], E. Meugnier [b], V. Mohan [a], M. Balasubramanyam [a,*]

[a] *Department of Cell and Molecular Biology, Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialties Centre, ICMR Centre for Advanced Research on Diabetes and WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control, Gopalapuram, Chennai 600086, India*
[b] *CarMeN Laboratory, Inserm 1060, Inra 1397, INSA, University of Lyon, Faculty of Medicine Lyon-Sud, 165 chemin du Grand-Revoyet, 69310 Pierre-Bénite, France*

ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form 27 June 2018
Accepted 11 August 2018
Available online xxx

Keywords:
Diabetes
Extracellular vesicles
MiRNA
Microalbumin
Nephropathy
Urinary

ABSTRACT

Aims. – MicroRNAs (miRNAs) from extracellular vesicles (EVs) have been proposed as promising biomarkers for a number of diseases. In this study, their potential as urine-based biomarkers of diabetic nephropathy (DN) was assessed.
Methods. – MiRNAs were profiled in urinary EVs from 160 fasting subjects with normal glucose tolerance (NGT) and in T2DM patients with either microalbumininuria (MIC) or macroalbuminuria (MAC).
Results. – A total of 73 miRNAs detected in urinary EVs (NGT) were predicted to target important functions for kidney homoeostasis, thereby validating their use as indicators of kidney dysfunction. Indeed, a urinary EV miRNA signature was found to comprise increased levels of let-7i-3p, miR-24-3p and miR-27b-3p, and decreased levels of miR-15b-5p, to identify patients with MIC. ROC curve analysis confirmed this ability to identify MIC in normo-albuminuria T2DM (T2DM-NA) patients and to differentiate between MAC and T2DM patients. These miRNAs were also predicted to target protein networks involved in the Wnt/β-catenin signalling cascade, activin receptor signalling and cell differentiation/proliferation, and correlated with eGRF, HbA_{1c}, serum creatinine, urea, albumin and blood pressure. Concentrations of miR-30a-5p were specifically modified in urinary EVs from patients with MAC, but not MIC, suggesting that miR-30a-5p could be related to severe kidney damage.
Conclusion. – Urinary EV miRNAs correlate with the degree of MIC. As they are also thought to regulate pathways that are targets of pharmacological agents to prevent DN (reticulum stress, activin receptors), they may also serve as non-invasive 'liquid biopsies' to stratify patients at risk of developing MAC and to monitor treatment efficacy.

Introduction

Diabetes is a leading cause of chronic kidney disease worldwide, and accounts for 31–40% of end-stage renal disease cases in India [1,2]. While longstanding type 2 diabetes mellitus (T2DM) and poor glycaemic control are risk factors for diabetic nephropathy (DN), South Asian ethnicity has also been linked to a relatively greater susceptibility to this complication; in fact, there are significant differences in the epidemiology of microvascular complications between South Asians and people of other races [3,4]. Hyperglycaemia, hypertension and genetic predisposition are among the significant risk factors that affect kidney glomeruli, arterioles, tubules and interstitium. Micro-albuminuria (MIC) is considered the gold standard indicator of DN, yet it poses certain specificity and sensitivity concerns. Its predictive powers are limited, as structural changes in the glomerular basement membrane and renal vasculature may appear before the onset of MIC [5,6]. Moreover, clinical factors unrelated to DN can affect MIC status [7] and, recently, it was observed that ≤ 30% of DN cases can arise in the absence of obvious MIC [8,9]. Therefore, there

* Corresponding author. Department of Cell and Molecular Biology, Madras Diabetes Research Foundation, Gopalapuram, Chennai 600086, India.
E-mail address: baluglobaldiab@gmail.com (M. Balasubramanyam).

https://doi.org/10.1016/j.diabet.2018.08.004
1262-3636/© 2018 Published by Elsevier Masson SAS.

PLOS | ONE

RESEARCH ARTICLE

Circulating MiRNAs of 'Asian Indian Phenotype' Identified in Subjects with Impaired Glucose Tolerance and Patients with Type 2 Diabetes

Paramasivam Prabu[1], Sophie Rome[2], Chandrakumar Sathishkumar[1], Sankaramoorthy Aravind[1], Balakumar Mahalingam[1], Coimbatore Subramanian Shanthirani[1], Caroline Gastebois[2], Audrey Villard[2], Viswanathan Mohan[1], Muthuswamy Balasubramanyam[1]*

1 Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control & IDF Centre of Education, Gopalapuram, Chennai-600086, India, **2** CarMeN Laboratory (INSERM 1060, INRA 1397, INSA), University of Lyon, Faculté de Médecine Lyon-Sud, Chemin du Grand Revoyet, 69600, Oullins, France

* diasignal@gmail.com

OPEN ACCESS

Citation: Prabu P, Rome S, Sathishkumar C, Aravind S, Mahalingam B, Shanthirani CS, et al. (2015) Circulating MiRNAs of 'Asian Indian Phenotype' Identified in Subjects with Impaired Glucose Tolerance and Patients with Type 2 Diabetes. PLoS ONE 10(5): e0128372. doi:10.1371/journal.pone.0128372

Academic Editor: Ratna B. Ray, SAINT LOUIS UNIVERSITY, UNITED STATES

Received: February 14, 2015

Accepted: April 26, 2015

Published: May 28, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Authors acknowledge the financial support from the Department of Biotechnology (DBT), Government of India. This work was also supported by grants from the French Fondation pour la Recherche Médicale (FRM-20101220456) and is supported by the French—Indian medical research cooperation program between INSERM and ICMR. The authors thank the study participants, and the clinical assistants and administrative personnel at the

Abstract

Several omics technologies are underway worldwide with an aim to unravel the pathophysiology of a complex phenotype such as type 2 diabetes mellitus (T2DM). While recent studies imply a clinically relevant and potential biomarker role of circulatory miRNAs in the etiology of T2DM, there is lack of data on this aspect in Indians—an ethnic population characterized to represent 'Asian Indian phenotype' known to be more prone to develop T2DM and cardiovascular disease than Europeans. We performed global serum miRNA profiling and the validation of candidate miRNAs by qRT-PCR in a cohort of subjects comprised of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and patients with T2DM. Our study revealed 4 differentially expressed miRNAs (miR-128, miR-130b-3p, miR-374a-5p, miR-423-5p) in subjects with IGT and T2DM patients compared to control subjects. They were positively or negatively correlated to cholesterol levels, HbA_{1C}, HOMA-IR and fasting insulin. Interestingly, circulating level of miR-128 and miR-130b-3p were also altered in serum of diet-induced diabetic mice compared to control animals. Among the altered circulating miRNAs, miR-128 had never been described in previous studies/populations and appeared to be a 'New Lead' in Indians. It was positively correlated with cholesterol both in prediabetic subjects and in diet-induced diabetic mice, suggesting that its increased level might be associated with the development of dyslipedemia associated with T2DM. Our findings imply directionality towards biomarker potential of miRNAs in the prevention/diagnosis/treatment outcomes of diabetes.

Molecular and Cellular Biochemistry (2019) 458:171–183
https://doi.org/10.1007/s11010-019-03540-9

Novel insights of elevated systemic levels of bisphenol-A (BPA) linked to poor glycemic control, accelerated cellular senescence and insulin resistance in patients with type 2 diabetes

Avinash Soundararajan[1,2] · Paramasivam Prabu[1] · Viswanathan Mohan[1] · Yann Gibert[2,3] · Muthuswamy Balasubramanyam[1]

Received: 26 December 2018 / Accepted: 12 April 2019 / Published online: 20 April 2019

Abstract

There is a striking interaction of genes and environment in the etiology of type 2 diabetes mellitus (T2DM). While endocrine disrupting chemicals (EDCs) like bisphenol-A (BPA) have received special attention for their mechanistic role in metabolic disruption, there is a lack of clinically relevant data on BPA levels in Asian Indians, a population which is more susceptible to type 2 diabetes mellitus (T2DM) and cardiovascular diseases. Therefore, we measured systemic levels of BPA in patients with T2DM compared to individuals with normal glucose tolerance ($n = 30$ each). Serum BPA levels were estimated using ELISA kit, and biochemical determinations were done by standard protocols. Peripheral blood mononuclear cells (PBMCs) were used to profile the gene expression alterations with special reference to inflammation, estrogen receptors, and cellular senescence in these subjects. Serum levels of BPA were significantly higher in patients with T2DM compared to control individuals and positively correlated to poor glycemic control and insulin resistance. Patients with T2DM exhibited significantly elevated mRNA levels of senescence (GLB1, p16, p21, and p53) and inflammatory (IL6 and TNF-α) markers, shortened telomeres as well as elevated levels of estrogen-related receptor gamma (ERRγ), a recently identified receptor for BPA. BPA levels were positively correlated to senescence indicators, inflammatory markers and ERRγ and negatively correlated to telomere length. Our study is the first data in the clinical diabetes setting to demonstrate an association of increased BPA levels with cellular senescence, proinflammation, poor glycemic control, insulin resistance, and shortened telomeres in patients with T2DM.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11010-019-03540-9) contains supplementary material, which is available to authorized users.

✉ Muthuswamy Balasubramanyam
balusignal@gmail.com; baluglobaldiab@gmail.com

[1] Department of Cell and Molecular Biology, Madras Diabetes Research Foundation & Dr. Mohan's Diabetes Specialties Centre, ICMR-Centre for Advanced Research on Diabetes, Gopalapuram, Chennai 600086, India

[2] Metabolic Research Unit, Metabolic Genetic Diseases Laboratory, School of Medicine, Deakin University, 75 Pigdons Road, Waurn Ponds, VIC 3216, Australia

[3] Present Address: Department of Cell and Molecular Biology, The University of Mississippi Medical Center, Jackson, MS 39216-4505, USA

Clinical Biochemistry xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Convergence of adipocyte hypertrophy, telomere shortening and hypoadiponectinemia in obese subjects and in patients with type 2 diabetes

Finny Monickaraj [a], Kuppan Gokulakrishnan [a], Paramasivam Prabu [a], Chandrakumar Sathishkumar [a], Ranjit Mohan Anjana [a], Janavikula Sankaran Rajkumar [b], Viswanathan Mohan [a], Muthuswamy Balasubramanyam [a,*]

[a] Madras Diabetes Research Foundation & Dr. Mohan's Diabetes Specialities Centre, Chennai, India
[b] LifeLine Multi-Speciality Hospitals, Chennai, India

ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 3 July 2012
Accepted 13 July 2012
Available online xxxx

Keywords:
Adipocyte hypertrophy
Telomere shortening
Hypoadiponectinemia
Metabolic obesity
Type 2 diabetes

ABSTRACT

Objective: Although telomere shortening has been linked with type 2 diabetes and most variables of adiposity, a shortcoming of such studies is the measurement of telomere length in leukocytes. Therefore, we tested the association among adipocyte cell size, telomere length (both subcutaneous and visceral adipose tissue) and systemic levels of adiponectin in obese subjects and patients with type 2 diabetes compared to control subjects.

Methods: Human subcutaneous and visceral adipose tissues were obtained from the subjects who have undergone bariatric surgery or other abdominal surgeries. The study groups comprised: i) control subjects, ii) type 2 diabetes patients, iii) obese subjects without diabetes and iv) obese subjects with diabetes. Adipocyte cell size was measured by histological staining. Adiponectin levels were measured by ELISA. Telomere length was determined by Real-time PCR and lipid peroxidation was assessed by fluorimetry.

Results: Compared to control subjects, adipocyte size (both subcutaneous and visceral) from obese, diabetic and obese–diabetic subjects was significantly larger [$p<0.001$]. Individuals with adipose hypertrophy also exhibited shortened telomeres and hypoadiponectinemia. Pearson correlation analysis revealed that both visceral and subcutaneous fat cell size showed a positive correlation with FBS, HbA1c, HOMA-IR, LDL, total cholesterol, triglycerides and negatively correlated with HDL and adiponectin. Regression analysis revealed that the association between shortened telomeres and hypoadiponectinemia was lost when adjusted for adipocyte cell size.

Conclusion: Adipocyte hypertrophy appears to be strongly associated with shortened telomeres, hypoadiponectinemia and poor glycemic and lipid control. Interestingly, these molecular alterations seen in lean diabetics reflect a state of 'metabolic obesity'.

1. Introduction

South Asians are at the higher risk of getting obesity related-non communicable diseases (OR-NCDs) compared with white Caucasian counter parts, which include insulin resistance, the metabolic syndrome, type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). The main reason for this accelerated increase in number of OR-NCDs is the rapid change in diet and more sophisticated life style [1]. Recent evidence also suggests that South Asians develop alterations in metabolic risk factors such as glucose, insulin, lipid levels and adipokines at significantly lower body mass indices than white Caucasians [2]. When introspecting the above statements, it is very much evident that there is a potential involvement of adipose tissue. It has been recently recognized that adipose cells have important endocrine function whereby they play a central role in the pathogenesis of diabetes and obesity. An increased proportion of visceral adipose tissue (VAT) is frequently reported to be associated with various metabolic diseases including type 2 diabetes [3]. In contrast with VAT, there is less consensus regarding the association between abdominal and peripheral subcutaneous adipose tissue (SAT) with disease risk, and both negative and positive associations have been reported [4]. Recent studies emphasize that adipocyte size is of importance in determining metabolic (i.e., lipolytic and lipogenic activities) and endocrine (i.e., leptin and adiponectin release) functions. Increased adipocyte size has been shown to predict the incidence of type 2 diabetes in women [5]. Moreover, adipocyte hypertrophy per se has been reported to be related to insulin resistance in type 2 diabetic patients independent of

Abbreviations: OR-NCDs, obesity related-non communicable diseases; T2DM, type 2 diabetes mellitus; CHD, coronary heart disease; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; BMI, Body mass index; LDL, Low density lipoprotein; HbA1c, Glycated hemoglobin; HOMA-IR, homeostasis assessment model-insulin resistance; MDA, Malonodialdehyde; TBARS, thiobarbituric acid reactive substances.

* Corresponding author at: Dept. of Cell and Molecular Biology, Madras Diabetes Research Foundation, 4, Conran Smith Road, Gopalapuram, Chennai-600 086, India. Fax: +91 44 28350935.
E-mail addresses: balusignal@gmail.com, drbalu@mdrf.in (M. Balasubramanyam).
URL: http://www.mdrf.in/ (M. Balasubramanyam).

0009-9120/$ – see front matter © 2012 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2012.07.097

Mol Cell Biochem (2012) 365:343–350
DOI 10.1007/s11010-012-1276-0

Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes

Finny Monickaraj · Sankaramoorthy Aravind · Kuppan Gokulakrishnan · Chandrakumar Sathishkumar · Paramasivam Prabu · Durai Prabu · Viswanathan Mohan · Muthuswamy Balasubramanyam

Received: 21 December 2011 / Accepted: 24 February 2012 / Published online: 13 March 2012

Abstract Although shortened telomeres were shown associated with several risk factors of diabetes, there is lack of data on their relationship with mitochondrial dysfunction. Therefore, we compared the relationship between telomere length and mitochondrial DNA (mtDNA) content in patients with type 2 diabetes mellitus (T2DM; $n = 145$) and in subjects with normal glucose tolerance (NGT; $n = 145$). Subjects were randomly recruited from the Chennai Urban Rural Epidemiology Study. mtDNA content and telomere length were assessed by Real-Time PCR. Malonodialdehyde, a marker of lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) using fluorescence methodology. Adiponectin levels were measured by radioimmunoassay. Oxidative stress as determined by lipid peroxidation (TBARS) was significantly ($p < 0.001$) higher in patients with T2DM compared to NGT subjects. In contrast, the mean telomere length, adiponectin and mtDNA content were significantly ($p < 0.001$) lower in patients with T2DM compared to NGT subjects. Telomere length was positively correlated with adiponectin, HDL, mtDNA content and good glycemic/lipid control and negatively correlated with adiposity and insulin resistance. On regression analysis, shortened telomeres showed significant association with T2DM even after adjusting for waist circumference, insulin resistance, triglyceride, HDL, adiponectin, mtDNA & TBARS. mtDNA depletion showed significant association with T2DM after adjusting for waist circumference and adiponectin but lost its significance when further adjusted for telomere length, TBARS and insulin resistance. Our study emphasizes the clustering of accelerated aging features viz., shortened telomeres, decreased mtDNA content, hypoadiponectinemia, low HDL, and increased oxidative stress in Asian Indian type 2 diabetes patients.

Keywords Telomere shortening · mtDNA depletion · Oxidative stress · Type 2 diabetes

Introduction

There is an upsurge in the prevalence of age-related diseases such as type 2 diabetes mellitus (T2DM) globally and more importantly in developing countries like India. Recent studies imply that regulation of aging and energy homeostasis share similar molecular pathways [1]. Many of the genes recently discovered that can be manipulated to slow the aging process belong to pathways involved in the control of metabolism. Energy homeostasis dysregulation occurs during the aging process and this appears to occur at an accelerated way in metabolic diseases like type 2 diabetes. Diabetes mellitus has recently been recognized as a cause of accelerated aging [2]. As the understanding of the metabolic syndrome has evolved, it has been recognized that the interaction of a panoply of factors in the presence of insulin resistance results in accelerated aging. The theory of aging [3] claims that the main place of production of free radicals (oxidative stress) is in mitochondria and this leads to mitochondrial DNA (mtDNA) damage and

F. Monickaraj · S. Aravind · K. Gokulakrishnan · C. Sathishkumar · P. Prabu · D. Prabu · V. Mohan · M. Balasubramanyam (✉)
Department of Cell and Molecular Biology, Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control, IDF Centre of Education, Gopalapuram, Chennai 600 086, Tamilnadu, India
e-mail: balusignal@gmail.com; drbalu@mdrf.in

Mol Cell Biochem (2011) 351:197–205
DOI 10.1007/s11010-011-0727-3

Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes

M. Balasubramanyam · S. Aravind · K. Gokulakrishnan · P. Prabu · C. Sathishkumar · H. Ranjani · V. Mohan

Received: 9 November 2010 / Accepted: 10 January 2011 / Published online: 20 January 2011

Abstract Type 2 diabetes patients exhibit subclinical inflammation but the regulatory mechanisms are poorly understood. We sought to evaluate the role of miR-146a expression along with its downstream proinflammatory signals in relation to glycemic control and insulin resistance. Study subjects ($n = 20$ each) comprised of clinically well characterized Type 2 diabetes patients and control non-diabetic subjects. miRNA and mRNA expression levels were probed in peripheral blood mononuclear cells (PBMC) by Real-time RT-PCR and plasma levels of TNFα and IL-6 were measured by ELISA. The miR-146a expression levels were significantly decreased in PBMCs from patients with Type 2 diabetes compared to control subjects. Among the target genes of miR-146a, TRAF-6 mRNA expression was significantly increased in patients with Type 2 diabetes while there was no significant difference in the mRNA levels of IRAK1 in the study groups. In contrast, there were significantly increased levels of NFκB expression in patients with Type 2 diabetes. There was an increased trend in the levels of TNFα and IL-6 mRNA in patients with type 2 diabetes. While SOCS-3 mRNA levels increased, plasma TNFα and IL-6 levels were also significantly higher in patients with type 2 diabetes. miR-146a expression was negatively correlated to glycated hemoglobin, insulin resistance, TRAF6, and NFκB mRNA levels and circulatory levels of TNFα and IL-6. Reduced miR-146a levels are associated with insulin resistance, poor glycemic control, and several proinflammatory cytokine genes and circulatory levels of TNFα and IL-6 in Asian Indian Type 2 diabetic patients.

Keywords miRNA · miR-146a · Insulin resistance · Inflammation · Type 2 diabetes · Asian Indians

Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre [WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control & IDF Centre of Education], Gopalapuram, Chennai, India.

M. Balasubramanyam (✉) · S. Aravind · K. Gokulakrishnan · P. Prabu · C. Sathishkumar · H. Ranjani · V. Mohan
Department of Cell and Molecular Biology, Madras Diabetes Research Foundation, Gopalapuram, Chennai 600086, India
e-mail: balusignal@gmail.com; drbalu@mdrf.in

Introduction

Recent studies imply that obesity, Type 2 diabetes, and cardiovascular diseases share a metabolic *milieu* characterized by insulin resistance and chronic low-grade inflammation. Increased levels of markers and mediators of inflammation and acute-phase reactants such as fibrinogen, C-reactive protein (CRP), IL-6, TNFα, plasminogen activator inhibitor-1 (PAI-1), sialic acid, and white cell count correlate with incidence of Type 2 diabetes [1]. Our earlier studies have shown increased systemic markers of inflammation in both Type 2 diabetes and prediabetic states [2, 3]. Furthermore, increased gene expression of TNFα and IL-6 were observed in peripheral blood mononuclear cells (PBMCs) in subjects with impaired glucose tolerance and patients with type 2 diabetes [4, 5]. Although the mechanisms by which proinflammatory monocytes and lymphocytes could influence insulin resistance and obesity were well studied, there is lack of data on how intracellular pathways are compromised in chronic inflammation.

Micro RNAs (miRNAs) are ~22 nucleotide RNA molecules that represent a family of heterogeneous, evolutionarily conserved, regulatory RNAs which recognize the 3′ un-translated regions (3′UTRs) of specific messenger RNA (mRNA) targets. They regulate the post-transcriptional