World of Science: Journal on Modern Research Methodologies

Volume 2 Issue 10, Year 2023
ISSN: 2835-3072
https://univerpubl.com/index.php/woscience

Line-1 Elements Gene Expression and Some Physiological Parameters Association with Diabetes Mellitus

Wisam Abdan Wawi AL Abdullah
Al-Qadisiyah University

Article Information

Received: Aug 28, 2023
Accepted: Sep 20, 2023
Published: Oct 21, 2023

Keywords: *diabetes mellitus, line-1, gene expression, glucose, lipid profile.*

ABSTRACT

Line-1 element has been implicated in disease progression and carcinogenesis. It is uncertain how this element's dysregulation contributes to the onset of diabetes, however, it can involve genomic instability and abnormal gene regulation. This project sought to examine transcript expression of line-1 in blood cells of diabetes mellitus patients and whether the line-1 associated with metabolic markers. The results revealed that blood glucose level, HbA1c were increased significantly in diabetes patients than healthy ($p \leq 0.001$). Additionally, TC, TG, LDL and VLDL concentration were significantly evaluated in patients compare to control ($p \leq 0.05$, $p \leq 0.001$, respectively). However, HDL serum level was decreased in diabetic patients ($p \geq 0.05$). In the case line-1, the relative fold change ($2^{-\Delta\Delta CT}$), the finding revealed that line-1 expression was significantly increased by ($p \leq 0.001$) fold in diabetes mellitus. In conclusion, the line-1 was up regulated in the diabetes patients and it was accompanied by change of physiological parameters levels. The there was a positive correlation line-1 transcription with glucose and HbA1c concentrations, in addition to lipid profile including TC, TG and LDL showed a positive relationship with line-1 transcription. In contrast, there were negative correlation line-1 expression with HDL and VLDL. There were positive relationship between mRNA line-1 expression and HDL/LDL as well as LDL/HDL ratio.

Introduction: Line-1 comprise further than 17% of the genome of human and they have present biological activity within the genome of human (Kano *et al.*, 2009). It induces damage to the cell by causing an abnormal expression of its sequence and generating insertion mutagenesis, further prompting cell apoptosis (Belgnaoui *et al*., 2006), causing DNA breaks (Gasior *et al*., 2006) triggering genetic damage in addition to mutations (Ostertag and Kazazian Jr, 2001). The irregular activity of line-1 or insertion mutagenesis through line-1 is involved in numerous diseases of human-like cancer, multiple sclerosis, and Alzheimer's (Barrow *et al*., 2021; Larsen *et al.*, 2018), Apert syndrome, cystic fibrosis, hemophilia, neurofibromatosis, and hyperlipidemia (Beck *et al.*, 2011; Callinan and Batzer, 2006). line-1 elements activity is constrained via several processes, including methylation of DNA and post-transcriptional regulator (Zamudio *et al*., 2015). Its methylation is related to genomic instability that is linked with many diseases for example, cancer (Wolff *et al*., 2010), Alzheimer's (Jones, 1986), cardiovascular disorders, and diabetes mellitus (Zhong *et al*., 2016) blood lipids, fasting glucose, stroke and heart disease (Cash *et al.*, 2011a; Pearce *et al.*, 2012; Turcot *et al.*, 2012). Barouti *et al*. (2022) reported the correlation methylation of line-1 with metabolic elements like hypertension, blood glucose, and lipid disorder. Methylation of line-1 was reduced in diabetes patients compared with healthy individuals (Maria Martin-Nunez *et al.*, 2014). However, studies showed line-1 hypermethylation related to an elevated possibility of developing diabetes (Pearce *et al.*, 2012).

Ge *et al*. (2003) indicated that expression of line-1 was pointedly decreased in type II diabetes patients. It was suggested that the expression of line 1 could be an indicator of severe metabolic disorder. Recent reported revealed line-1 may control metabolism by inserting genes of metabolic. For example, insertions of line-1 in the carbohydrate kinase family gene (FGGY) lead to up-regulate arachidonic acid metabolism, glycerolipid metabolism, and cytochrome P450 (Zhang *et al*., 2019). Consequently, the current project purposed to explore whether mRNA expression of line-1 elements is related to diabetes mellitus and some physiological parameters.

Materials and methods: Diabetes mellitus patients attended to consultant clinic at the Diabetes Center in Al-Diwaniyah Teaching Hospital, Iraq. Patients clinically examined by consultant medical staff and confirmed by test fasting blood glucose level (FBG) is ≥ 7 mmol/l. HbA1c (glycated hemoglobin) 6.5% was by American Diabetes Association criteria (Horvath et al., 2010). Healthy (control) groups selected from healthcare units in Al-Diwaniyah Teaching Hospital. They had no clinical evidence or family history of diabetes mellitus, FBG < 6.1 mmol/) and HbA1c < 6.5%, or other diseases.

Blood collection: Blood samples from the individuals taken (healthy and patient). 10 ml of all subjects had their venous obtained while fasting. 2 ml of the blood added to the EDTA tube for mRNA isolation and HbA1c measurement. 3ml of the blood drawn into EDTA vacuum tubes. Blood samples centrifuged at 3000rpm at 4°C for 15 min to obtain the serum. Aliquots of the separated serum made and utilized to determine biochemical measurements. Blood serum stored at –80°C until used.

RNA purification and cDNA production: mRNA separated by adding 1ml Reagent of Trizol (Invitrogen) into blood samples by the producer's instructions. NanoDrop was utilized to evaluate RNA purity and concentration. Before cDNA synthesis, total RNA immediately treated to eliminate contaminating DNA. mRNA reversely transcribed cDNA utilizing a superscript II reverse transcriptase kit (Invitrogen).

RT-qPCR experments: RT-qPCR was achieved by LightCycler 480 equipment (Roche, Mannheim, Germany) by using bright green qPCR master mix kit (ABM) and oligonucleotide primers for line-1 and human GAPDH as an internal control of mRNA. Oligonucleotide primers were designed using web-based service and validated by the NCBI BLAST database as showed Line-1 forward sequence TGCGGAGAAATAGGAACACTTTT and reveal sequence TGAGGAA TCGCCACACTGACT. GAPDH: forward sequences GTCAAGGCTGAGAACGGGAA and reveals sequence TCGCCCCACTTGATTTTGGA. Gene expression was analyzed by deterring $2^{-\Delta\Delta Ct}$ and relative Fold Change (Livak and Schmittgen, 2001) the gene of interesting was normalized and calculated to the expression level of GAPDH to evaluate expression target gene.

Determination of physiological biomarkers: Testing was carried out as soon as the serum was separated using standard procedures. The enzymatic colorimetric approach was used to determine the serum glucose levels and the serum lipid profile (cholesterol, triglyceride, HDL, LDL and VLDL) by utilizing the BIOLABSkit (France). HbA1c concentration in whole blood was assessed using a colorimetric fluorescence immunoassay (FIA) kit from Boditech Medical.

Statistic evaluation: Graph Pad Prism 8.0 software (USA) was used to carry out the statistic analysis of the data. T-tests analysis was used to analysis the statistical variances between groups. Pearson's correlation coefficient was calculated among all physiological biomarkers and the gene expression in diabetes group. *P* value was measured statistically substantial of a comparison between groups.

Results: Line-1 was expressed at CT 15.24±2.62 in diabetes patients compared to 19.34±1.25 in healthy individuals, as shown in Table (1). The ΔCT values for Line-1 of diabetes patients were elevated significantly (p≤ 0.05) -10.84±1.56 compared to ΔCT of healthy -9.06 ±1.21. Using

ΔΔCT values that revealed -1.85, the various ΔCT values of Line-1 transcription for healthy and diabetes patients were calculated in Table (1). As demonstrated in Table (1) and Figure (1), folding change data showed that diabetes patients' line-1 mRNA is considerably (p≤0.001) increased when compared to healthy individuals (3.61).

Individuals	C_T GAPDH	C_T Line-1	ΔC_T	$\Delta\Delta C_T$	Fold change
Control	28.40±1.01	19.34±1.25	-9.06±1.21	-1.85	3.61
Patients	26.08±1.12	15.24±2.62	-10.84±1.56*		

Table (1) line-1 gene expression in human diabetes patients and normal humans. *Data are explained as mean± S.E.M. less CT or ΔCT level is increased gene expression, (*p≤0.05).*

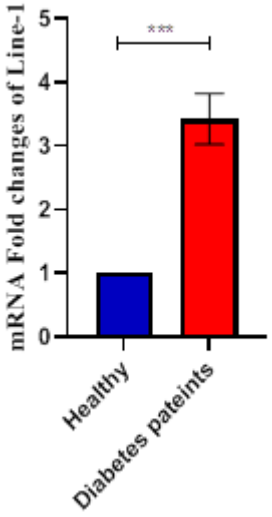

Figure (1) line-1 gene expression in diabetes patients and normal humans. Data are displayed as mean± S.E.M., (***p≤0.001).

The data exhibited glucose significantly increased (p≤0.001) in diabetic patients (223.80 ± 7.67 mg/dl,) compared with control (107.10 ± 4.07mg/dl). HbA1c significantly increased (p≤0.001) in diabetic patients (9.16 ± 0.35%) as compared with healthy (5.25 ± 0.14%), table (2). Additionally, data showed that serum TC levels in diabetic patients were evaluated significantly (p≤0.001) in comparison with controls (188.3±7.09 and 129.7±6.25, respectively). Similarly, there were significantly elevated serum diabetes patients' TG levels (231.6± 28.25, p≤0.05) compared to those of healthy people (144.0± 17.10). However, HDL levels significantly declined in the patient's group (22.05±0.49, p≤0.05) compared with the healthy group (28.96±2.96). In diabetes patients, LDL and VLDL were significantly increased (122.9±6.40 and 30.52±3.74, p≤0.05 respectively) when compared with healthy (97.92±9.70 and 18.43±1.78, respectively), table (2). There are no significant changes in HDL/LDL ratio in patients (-52.69±28.38, p≥0.05) compared to healthy. In contrast, LDL/HDL ratio showed significantly higher in patients (100.3±6.18,p≤0.01) than healthy group (58.99±10.30), table (2).

According to correlation analysis between line-1 expression and physiological parameters for diabetes mellitus patients as shown in Table (3), the results revealed that a positive correlation between expression of line-1 with glucose and HbA1c concentrations (r = 0.267 and r = 0.255, respectively). Lipid profiles including TC, TG, and LDL presented a positive correlation line-1 expression (r = 0.233, r = 0.138, and r = 0.109, respectively), table (3). However, the results showed a negative association between line-1 expressions with HDL and VLDL (r = -0.456 and r= -0.406, respectively), see table (3). Data analysis showed a positive correlation line-1 with HDL/LDL ratio (r = 0.233) as well as LDL/HDL ratio (r = 0.044), table (3).

Biochemical parameters	Healthy subjects	Diabetes pateints	p-value
FBG	107.1±4.07	223.8±7.67	p≤0.001
HbA1c (%)	5.25±0.149	9.16±0.35	p≤0.001
Cholesterol	129.7±6.25	188.3±7.09	p≤0.001
Triglyceride	144.0±17.10	231.6±28.25	p≤0.0422
HDL	28.96±28.96	22.05±0.49	p≤0.0392
LDL	97.92±9.70	122.9±6.40	p≤0.0427
VLDL	18.43±1.78	30.52±3.74	p≤0.0260
HDL/LDL	-40.85±20.54	-52.69±28.38	p≤0.1637
LDL/HDL	58.99±10.30	100.3±6.18	p≤0.0018

Table (2) physiological parameters of patients with diabetes mellitus and normal humans.
Data are displayed as mean± S.E.M, (p≥0.05, p≤0.05, p≤0.01, p≤0.001).

physiological parameters	*r*	95% confidence interval	p-value
Blood glucose	0.267	-0.333 to 0.713	p≤0.3779
HbA1c (%)	0.255	-0.343 to 0.706	p≤0.3996
Cholesterol	0.233	-0.364 to 0.695	p≤0.4430
Triglyceride	0.138	-0.5790 to 0.734	p≤0.7227
HDL	-0.456	-0.816 to 0.158	p≤0.1356
LDL	0.109	-0.644 to 0.755	p≤0.7961
VLDL	-0.406	-0.795 to 0.218	p≤0.1895
HDL/LDL	0.233	-0.393 to 0.711	p≤0.4660
LDL/HDL	0.044	-0.543 to 0.602	p≤0.8918

Table (3) Correlation Line-1 expression and some physiological parameters of patients with diabetes mellitus.

Discussion: Resulting showed significantly higher levels of glucose, HbA1c, cholesterol, triglyceride, LDL, and VLDL in diabetes patients in contrast with healthy. However, HDL level was decreased in diabetes patients compared with healthy. Quantitative PCR data found that in diabetes patients, line-1 significantly expressed than control. De-repressed line-1, however, serves as a diagnostic indicator for some diseases and a driver of numerous diseases (Pedersen and Zisoulis, 2016). High-level expression of line-1 is linked to cellular repair, DNA damage, apoptosis, stress reactions, and tumor progression (Morrish *et al.*, 2007; Sinibaldi-Vallebona *et al*., 2006). It discovered a substantial positive association between line 1 and glucose, which was most noticeable in people with diabetes. The results of the current investigation demonstrated a favorable correlation between line-1 expression with glucose level and HbA1c. A significant relationship between serum glucose concentration and line-1 methylation was reported in a discussion (Pearce *et al.*, 2012). However, studies showed that line-1 methylation was adversely related to blood sugar levels (Turcot *et al*., 2012). The precise mechanism is yet unknown. The syndrome known as diabetes mellitus is at risk due to elevated glucose levels, thus even though the pathway underlying this link is unknown. Line-1's activity as a retrotransposable sequence that may influence gene expression and cause genomic changes via a variety of pathways is increased by hypomethylation (Schulz *et al.*, 2006). According to earlier research, oxidative stress may service line-1 expression while disrupting the expression of genes implicated repair of DNA (Giorgi *et al.*, 2011; Wongpaiboonwattana *et al.*, 2013). One of the potential causes of chronic inflammation may be oxidative stress-induced line-1 activation (Xiao-Jie *et al.*, 2016). Diabetes's high blood sugar levels are thought to be a significant component in the development of oxidative stress and inflammation (Wojcik *et al.*, 2020). Oxidative stress brought on by inflammation in diabetes may promote line-1 expression. It is yet unknown how increased line-1 activity may cause pro-inflammatory signals to be induced and maintained. An increase in

oxidative stress, which causes genomic instability and DNA damage, may activate line-1, which may be essential for chromosomal and genomic integrity. Therefore, these components might influence the pathophysiology of diabetes. There is currently no evidence that line-1 expression and diabetes in people with diabetes mellitus are related in any way. Our comprehension of the connection between transposons elements-mediated disease and the etiology of diabetes may be improved by this information. According to Maria Martin-Nunez *et al.* (2014), , people with T2D had lower methylation of line-1 in their blood cells than people who were not diabetic. In contrast, line-1 hypermethylation, lower expression, has been related with evaluated levels of blood glucose, cholesterol, triglycerides, and LDL-cholesterol in diabetes type II (Pearce *et al.*, 2012). Wu and colleagues found that patients with diabetes type II had considerably evaluated line-1 methylation compare to healthy controls (Wu *et al.*, 2017). It discovered that insulin resistance was substantially linked with hypermethylation of lein-1 element in leukocytes from those who had type II diabetes (Zhao *et al*., 2012). We also investigated the link between line-1 expression levels with glucose and lipid dysregulation. Our results revealed no statistically significant correlation between the line-1 gene and HDL levels. However, we discovered a robust correlation between LDL concentration and the line-1 gene. The association between expression line 1 and the lipid indicators was confirmed by the vast majority of the studies that were examined (Braun *et al.*, 2017; Dayeh *et al*., 2016; Kriebel *et al*., 2016; Walaszczyk *et al.*, 2018). Levels of the line-1 gene were displayed to be linked with HDL-C and LDL-C. Increasing expression of line-1 was correlated with lower concetration of HDL and higher LDL values (Cash *et al.*, 2011b). Lipoproteins in peripheral blood likely have an impact on methylation of line-1, potentially contributing to the increased line-1 expression and its hypomethylation found in atherosclerotic (Hiltunen *et al*., 2002; Yideng *et al.*, 2007) This research highlighted line-1potential function as a predictor of associated metabolic problems or the likelihood of promoting type 2 diabetes. Methylation of line-1 is related to elevated LDL and decreased HDL concentrations, as well as to several blood-based metabolic indicators (Pearce *et al.*, 2012). According to Turcot *et al*. (2012), they found that line 1 has also been shown to be connected to cholesterol and blood sugar levels. Studies showed that line-1 hypermethylation compared to hypomethylation was connected with a greater risk of metabolic disorders (Maria Martin-Nunez *et al*., 2014). It was discovered that methylation of the line-1 was negatively linked by HDL, the HDL/LDL ratio, and favorably related to LDL, total cholesterol, and triglycerides (Pearce *et al*., 2012). Additionally, research revealed a weak and negative connection with HDL and a positive association was identified between line-1 methylation and the ratio of LDL/HDL (Walaszczyk *et al*., 2018).

Conclusion: line-1 is significantly up- regulated in diabetes mellitus patients. Glucose, HbA1c, and lipid profile are significant correlates with line-1 expression. It suggested that physiological parameters are linked to greater levels of line-1 expression. It is unknown how line-1 expression and diabetes mellitus are related. A longitudinal study would be an intriguing method to learn more about the connections between diabetes and line 1 in the progression of the disease, especially given that diabetes disease is currently one of the main causes of death accurse in the world.

References

1. Barouti Z, Heidari-Beni M, Shabanian-Boroujeni A, Mohammadzadeh M, Pahlevani V, Poursafa P, Mohebpour F and Kelishadi R (2022) Effects of DNA methylation on cardiometabolic risk factors: a systematic review and meta-analysis. *Archives of Public Health* **80**:1-16.
2. Barrow TM, Doo NW, Milne RL, Giles GG, Willmore E, Strathdee G and Byun H-M (2021) Analysis of retrotransposon subfamily DNA methylation reveals novel early epigenetic changes in chronic lymphocytic leukemia. *haematologica* **106**:98.

3. Beck CR, Garcia-Perez JL, Badge RM and Moran JV (2011) LINE-1 elements in structural variation and disease. *Annual review of genomics and human genetics* **12**:187-215.

4. Belgnaoui SM, Gosden RG, Semmes OJ and Haoudi A (2006) Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells. *Cancer cell international* **6**:1-10.

5. Braun KV, Dhana K, de Vries PS, Voortman T, van Meurs JB, Uitterlinden AG, Hofman A, Hu FB, Franco OH and Dehghan A (2017) Epigenome-wide association study (EWAS) on lipids: the Rotterdam Study. *Clinical epigenetics* **9**:1-11.

6. Callinan P and Batzer M (2006) Retrotransposable elements and human disease. *Genome and disease* **1**:104-115.

7. Cash HL, McGarvey ST, Houseman EA, Marsit CJ, Hawley NL, Lambert-Messerlian GM, Viali S, Tuitele J and Kelsey KT (2011a) Cardiovascular disease risk factors and DNA methylation at the LINE-1 repeat region in peripheral blood from Samoan Islanders. *Epigenetics* **6**:1257-1264.

8. Cash HL, McGarvey ST, Houseman EA, Marsit CJ, Hawley NL, Lambert-Messerlian GM, Viali S, Tuitele J and Kelsey KTJE (2011b) Cardiovascular disease risk factors and DNA methylation at the LINE-1 repeat region in peripheral blood from Samoan Islanders. **6**:1257-1264.

9. Dayeh T, Tuomi T, Almgren P, Perfilyev A, Jansson P-A, de Mello VD, Pihlajamäki J, Vaag A, Groop L and Nilsson E (2016) DNA methylation of loci within ABCG1 and PHOSPHO1 in blood DNA is associated with future type 2 diabetes risk. *Epigenetics* **11**:482-488.

10. Gasior SL, Wakeman TP, Xu B and Deininger PL (2006) The human LINE-1 retrotransposon creates DNA double-strand breaks. *Journal of molecular biology* **357**:1383-1393.

11. Ge S, Ren Y and Xu R (2003) Study of functional L1 retrotransposon in human type 2 diabetes susceptibility loci. *Zhonghua yi xue yi Chuan xue za zhi= Zhonghua Yixue Yichuanxue Zazhi= Chinese Journal of Medical Genetics* **20**:287-291.

12. Giorgi G, Marcantonio P and Del Re B (2011) LINE-1 retrotransposition in human neuroblastoma cells is affected by oxidative stress. *Cell and tissue research* **346**:383-391.

13. Hiltunen MO, Turunen MP, Häkkinen TP, Rutanen J, Hedman M, Mäkinen K, Turunen A-M, Aalto-Setalä K and Ylä-Herttuala S (2002) DNA hypomethylation and methyltransferase expression in atherosclerotic lesions. *Vascular medicine* **7**:5-11.

14. Jones PA (1986) DNA methylation and cancer. *Cancer research* **46**:461-466.

15. Kano H, Godoy I, Courtney C, Vetter MR, Gerton GL, Ostertag EM and Kazazian HH (2009) L1 retrotransposition occurs mainly in embryogenesis and creates somatic mosaicism. *Genes & development* **23**:1303-1312.

16. Kriebel J, Herder C, Rathmann W, Wahl S, Kunze S, Molnos S, Volkova N, Schramm K, Carstensen-Kirberg M and Waldenberger M (2016) Association between DNA methylation in whole blood and measures of glucose metabolism: KORA F4 study. *PloS one* **11**:e0152314.

17. Larsen PA, Hunnicutt KE, Larsen RJ, Yoder AD and Saunders AM (2018) Warning SINEs: Alu elements, evolution of the human brain, and the spectrum of neurological disease. *Chromosome Research* **26**:93-111.

18. Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods* **25**:402-408.

19. Maria Martin-Nunez G, Rubio-Martín E, Cabrera-Mulero R, Rojo-Martínez G, Olveira G, Valdés S, Soriguer F, Castano L and Morcillo S (2014) Type 2 diabetes mellitus in relation to global LINE-1 DNA methylation in peripheral blood: a cohort study. *Epigenetics* **9**:1322-1328.

20. Morrish TA, Garcia-Perez JL, Stamato TD, Taccioli GE, Sekiguchi J and Moran JV (2007) Endonuclease-independent LINE-1 retrotransposition at mammalian telomeres. *Nature* **446**:208-212.

21. Ostertag EM and Kazazian Jr HH (2001) Biology of mamalian L1 retrotransposons. *Annual review of genetics* **35**:501.

22. Pearce MS, McConnell JC, Potter C, Barrett LM, Parker L, Mathers JC and Relton CL (2012) Global LINE-1 DNA methylation is associated with blood glycaemic and lipid profiles. *International journal of epidemiology* **41**:210-217.

23. Pedersen IM and Zisoulis DG (2016) Transposable elements and miRNA: Regulation of genomic stability and plasticity. *Mobile genetic elements* **6**:824-831.

24. Schulz W, Steinhoff C and Florl A (2006) Methylation of endogenous human retroelements in health and disease. *DNA Methylation: Development, Genetic Disease and Cancer*:211-250.

25. Sinibaldi-Vallebona P, Lavia P, Garaci E and Spadafora C (2006) A role for endogenous reverse transcriptase in tumorigenesis and as a target in differentiating cancer therapy. *Genes, Chromosomes and Cancer* **45**:1-10.

26. Turcot V, Tchernof A, Deshaies Y, Pérusse L, Bélisle A, Marceau S, Biron S, Lescelleur O, Biertho L and Vohl M-C (2012) LINE-1 methylation in visceral adipose tissue of severely obese individuals is associated with metabolic syndrome status and related phenotypes. *Clinical epigenetics* **4**:1-8.

27. Walaszczyk E, Luijten M, Spijkerman AM, Bonder MJ, Lutgers HL, Snieder H, Wolffenbuttel BH and van Vliet-Ostaptchouk JV (2018) DNA methylation markers associated with type 2 diabetes, fasting glucose and HbA1c levels: a systematic review and replication in a case–control sample of the Lifelines study. *Diabetologia* **61**:354-368.

28. Wojcik M, Krawczyk M, Zieleniak A, Mac Marcjanek K and Wozniak LA (2020) Associations of high blood sugar with oxidative stress and inflammation in patients with type 2 diabetes, in *Dietary sugar, salt and fat in human health* pp 305-323, Elsevier.

29. Wolff EM, Byun H-M, Han HF, Sharma S, Nichols PW, Siegmund KD, Yang AS, Jones PA and Liang G (2010) Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. *PLoS genetics* **6**:e1000917.

30. Wongpaiboonwattana W, Tosukhowong P, Dissayabutra T, Mutirangura A and Boonla C (2013) Oxidative stress induces hypomethylation of LINE-1 and hypermethylation of the RUNX3 promoter in a bladder cancer cell line. *Asian Pacific Journal of Cancer Prevention* **14**:3773-3778.

31. Wu Y, Cui W, Zhang D, Wu W and Yang Z (2017) The shortening of leukocyte telomere length relates to DNA hypermethylation of LINE-1 in type 2 diabetes mellitus. *Oncotarget* **8**:73964.

32. Xiao-Jie L, Hui-Ying X, Qi X, Jiang X and Shi-Jie M (2016) LINE-1 in cancer: multifaceted functions and potential clinical implications. *Genetics in Medicine* **18**:431-439.

33. Yideng J, Jianzhong Z, Ying H, Juan S, Jinge Z, Shenglan W, Xiaoqun H and Shuren W (2007) Homocysteine-mediated expression of SAHH, DNMTs, MBD2, and DNA

hypomethylation potential pathogenic mechanism in VSMCs. *DNA and cell biology* **26**:603-611.

34. Zamudio N, Barau J, Teissandier A, Walter M, Borsos M, Servant N and Bourc'his D (2015) DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination. *Genes & development* **29**:1256-1270.
35. Zhang R, Zhang F, Sun Z, Liu P, Zhang X, Ye Y, Cai B, Walsh MJ, Ren X and Hao X (2019) LINE-1 Retrotransposition Promotes the Development and Progression of Lung Squamous Cell Carcinoma by Disrupting the Tumor-Suppressor Gene FGGYL1 Promotes LUSC Development by Disruption of FGGY. *Cancer Research* **79**:4453-4465.
36. Zhao J, Goldberg J, Bremner JD and Vaccarino V (2012) Global DNA methylation is associated with insulin resistance: a monozygotic twin study. *Diabetes* **61**:542-546.
37. Zhong J, Agha G and Baccarelli AA (2016) The role of DNA methylation in cardiovascular risk and disease: methodological aspects, study design, and data analysis for epidemiological studies. *Circulation research* **118**:119-131.