

Alu DNA Polymorphism of Human Tissue Plasminogen Activator (*tPA*) Gene in Diabetic Jordanian Patients

Salem R. Yasin^{1*}, Hussam H. AlHawari², Abeer A. Alassaf³, Maysa M. Khadra⁴,
Zainab A. Al-Mazaydeh¹, Ala'a F. Al-Emerieen⁵ and Lubna H. Tahtamouni¹

¹Department of Biology and Biotechnology, Faculty of Science, the Hashemite University, Zarqa, Jordan;

²Department of Internal Medicine, Faculty of Medicine, University of Jordan, Amman, Jordan;

³Department of Pediatric, Faculty of Medicine, University of Jordan, Amman, Jordan;

⁴Department of Obstetrics and Gynecology, Faculty of Medicine, University of Jordan, Amman, Jordan;

⁵Department of Service Courses, Faculty of Science, Zarqa Private University, Zarqa, Jordan

Received 8 September 2018; revised 6 November 2018; accepted 10 November 2018

ABSTRACT

Background: Hypercoagulability and hypofibrinolysis are among the symptoms exhibited by diabetic patients. Our study aimed to address the polymorphic nature of *Alu* DNA fragment in the human tissue plasminogen activator gene within diabetes mellitus (DM) Jordanian patients. **Methods:** Genomic DNA was isolated from 76 DM patients and 60 non-diabetic Jordanian individuals, and the *Alu* fragment was amplified using PCR. **Results:** The results showed that 80% of the non-diabetic Jordanian subjects were homozygotes for the deletion of the *Alu* fragment (*Alu*^{-/-}), 16.7% were homozygotes for its insertion (*Alu*^{+/+}), and 3.3% were heterozygotes (*Alu*^{+/-}). Besides, 36.8% of the diabetic patients exhibited the *Alu*^{-/-} or *Alu*^{+/-} genotype, and 26.3% were *Alu*^{+/+}. The *Alu*^{-/-} genotype occurred less frequently in the diabetic individuals. **Conclusion:** The high frequency of the *Alu*^{-/-} genotype constitutes a protective deletion with respect to DM within the normal subjects. **DOI: 10.29252/ibj.23.6.423**

Keywords: *Alu*, Diabetes mellitus, Polymorphism

Corresponding Author: Salem R. Yasin

The Hashemite University, Faculty of Science, Department of Biology and Biotechnology, Zarqa 13115, Jordan; P.O. Box: 150459; Tel.: (+96-25) 3903349; Fax: (+96-27) 97079332; E-mail: salemmaloul@yahoo.com

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia and caused by defects in insulin secretion, insulin function, or both^[1]. Based on etiology and pathology, DM has been classified into DM type 1 (T1DM) and DM type 2 (T2DM)^[2,3]. The major complication resulting from DM is related to the vascular system. Diabetic patients present symptoms of hypercoagulability and hypofibrinolysis; about 80% of diabetics die from thrombotic events^[4,5].

The fibrinolytic system is responsible for the dissolution of fibrin blood clot^[6]. The components of

this system include tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), and inhibitors, PA inhibitor-1 (PAI-1) and PAI-2. tPA and uPA catalyze the inactive pro-enzyme plasminogen into the dynamic plasmin. tPA is mainly involved in thrombolysis^[7-9]. Meanwhile, tPA is synthesized in the vascular endothelial cells and is released into the blood when stimulated^[10,11]. Its discharge, dispersion, complex formation with PAI-1 and release rate influence the tPA levels^[12-14]. However, only tPA release rate affects the thrombolytic potential of tPA^[15]. Hyperglycemia prevents the activity of the fibrinolytic system by stimulating the production of PAI-1. Abnormalities in the fibrinolytic system

precede the development of T2DM^[13,16], which was associated with PAI-1 increased concentration^[5,17]. Elevated levels of PAI-1 and tPA antigens and reduced levels of tPA have been observed in diabetes and metabolic syndrome (MetS)^[10,13].

The human tPA gene is located on chromosome 8p12-p11.2^[18], and one polymorphism, an *Alu* repeat polymorphism, has been found in intron 8 of this gene^[19,20]. Members of the *Alu* family are short (approximately 311 base pairs) interspersed DNA elements. *tPA* polymorphism exhibits three different genotypes: *Alu*^{+/+} and *Alu*^{-/-} homozygotes and *Alu*^{+/-} heterozygote, which are based on either the insertion (I) or deletion (D) of the *Alu* element. The *Alu*⁺ allele is the derived allele^[21]. Many populations have been reported to be dimorphic for the presence or absence of the *Alu* repeats^[22-25].

There are many investigations focused on the tPA levels and its possible association with certain clinical statuses, but few have been addressed the genotypic polymorphism of the *Alu* fragment of the *tPA* gene and its effect. The *tPA* gene *Alu* polymorphism has been observed to regulate the interaction between tPA and PAI-1, and the presence of the *Alu* repeats in both alleles of this gene (*Alu*^{+/+}) has been shown to associate with the elevated levels of plasma PAI-1 and tPA antigens^[12]. Furthermore, the *Alu* polymorphism has not been found to be involved in tPA production but rather in its release rate^[15]. Though this genotype has been implicated as a risk factor in T2DM and MetS^[13], an association between the I/D polymorphism and tPA synthesis or plasma levels has not been investigated yet^[26-28]. In this study, we intended to address the genotypic and allelic distributions and possible association of the *Alu* DNA diverseness in the *tPA* gene within DM Jordanian patients.

MATERIALS AND METHODS

Study subjects

In this study, 76 DM patients (26 T1DM and 50 T2DM) and 60 aged-matched healthy non-diabetic Jordanian individuals (glycated hemoglobin level [HbA1c] < 42 mmol/mol, fasting blood sugar < 100 mg/dL; data not shown) were recruited from Jordan University Hospital in Amman, Jordan from March 2016 to May 2017. Patients with impaired glucose tolerance, gestational diabetes, maturity onset diabetes of youth, or metabolic syndrome were excluded. All individuals gave their informed consents, and the study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, the University of Jordan, which conforms to the Declaration of Helsinki.

tPA genotyping

Peripheral blood (3 ml) was collected in EDTA tubes from each participant by venous puncture. DNA was extracted from 300 µL blood using a commercially available kit (Promega, USA). Total genomic DNA amplification was carried out as previously reported^[29]. Briefly, 0.3 µg of genomic DNA from both normal and DM patients was subjected to amplification by PCR in a 30 µl total volume reaction containing 1× Master Mix (0.5 U of Taq DNA polymerase, 0.2 mM of dNTPs, and 1.5 mM of MgCl₂; Promega, USA) and 0.2 µM each of 5'-flanking (GTAACCATTTAGTCCTCAGC TGTTCTCCT) and 3'-flanking (CCATGTAAGAGTA GAAGGAGACTCAGTCA) primers (the Midland Certified Reagent Co., USA). Amplification of DNA was performed in a MyCycler thermal cycler (BioRad, USA) at 96 °C for 2 min, followed by 35 cycles of denaturation (96 °C for 30 s), annealing at 65 °C for 30 s, and synthesis at 72 °C for 30 s. This process was followed by an extension step at 65 °C for 5 min. Amplicons were electrophoresed and visualized on 2% (w/v) agarose gel with 0.5 µg/ml of ethidium bromide. Individuals carrying the *tPA* *Alu* inserts were designated homozygotes as *Alu*^{+/+} and heterozygotes as *Alu*^{+/-}, as well as homozygotes for the absence of the insert as *Alu*^{-/-}.

Statistical analysis

The observed genotypes and allele frequencies were compared with those expected in order to verify the Hardy-Weinberg equilibrium. The Chi-square test and Fisher's exact test were performed for the polymorphism frequency using Statistica software, StatSoft Inc, Tulsa, OK, USA (version 10). A value of $p < 0.05$ was considered statistically significant.

RESULTS

DNA was successfully extracted from 60 normal non-diabetic subjects, 26 T1DM patients, and 50 T2DM patients (data not shown). The PCR amplification products of the 300 bp *Alu* region of *tPA* gene are shown in Figure 1.

The PCR amplification results indicated that all the three genotypes, *Alu*^{+/+} (600 bp), *Alu*^{+/-} (600/300 bp), *Alu*^{-/-} (300 bp) were observed in both study groups (normal Jordanian individuals and DM patients; Fig. 1 and Table 1). The highest genotype frequency in normal non-diabetic individuals was the *Alu*^{-/-} genotype at 80.0%, while both *Alu*^{-/-} and *Alu*^{+/-} genotypes were equally higher in the DM patients at 36.8% each. In DM patients, the *Alu*^{+/+} genotype percentage was 26.32%. The results in Table 1 showed a significant

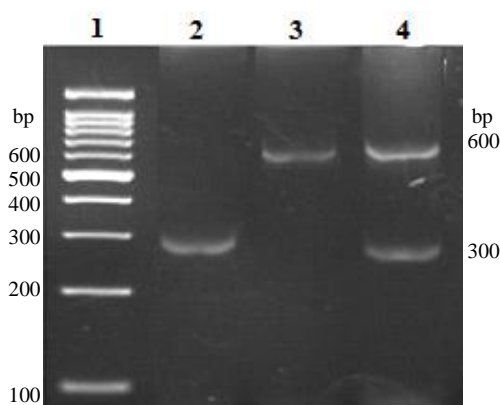


Fig. 1. Representative *tPA* *Alu* DNA amplification. The PCR products were electrophoresed and visualized with ethidium bromide. The 300-bp band indicates the absence of the *Alu* insert, while the 600-bp band shows the presence of the *Alu* insert. Lane 1, 100 bp DNA molecular weight marker; lane 2, *Alu*^{-/-} genotype; lane 3, *Alu*^{+/-} genotype; lane 4, *Alu*^{+/+} genotype.

Table 1. Statistical analysis of *Alu* genotypes and allelic distributions of tissue plasminogen activator (*tPA*) between normal non-diabetic (n = 60) and diabetic (DM [n = 76], T1DM [n = 26], and T2DM [n = 50]) Jordanian subjects

		Normal group % (n)	DM group % (n)	<i>p</i> value	Odds ratio 95% CI	<i>p</i> value
Genotype	<i>Alu</i> ^{-/-}	80.0 (48)	36.8 (28)	0.0001	Vs (<i>Alu</i> ^{+/+} + <i>Alu</i> ^{+/-}) = 9.63 2.56-30.89	0.0001
	<i>Alu</i> ^{+/-}	3.3 (2)	36.8 (28)	0.0001	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/+}) = 9.72 2.92-29.28	0.0001
	<i>Alu</i> ^{+/+}	16.7 (10)	26.3 (20)	0.1809	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/-}) = 8.79 3.23-23.41	0.001
Allele	<i>Alu</i> ⁻	0.82	0.55	0.0012		
	<i>Alu</i> ⁺	0.18	0.45	0.0012		
		Normal group % (n)	T1DM group % (n)	<i>p</i> < 0.05	Odds ratio 95% CI	<i>p</i> value
Genotype	<i>Alu</i> ^{-/-}	80.0 (48)	34.6 (9)	0.0001	Vs (<i>Alu</i> ^{+/+} + <i>Alu</i> ^{+/-}) = 0.02 0.001-0.29	0.0058
	<i>Alu</i> ^{+/-}	3.3 (2)	38.5 (10)	0.0001	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/+}) = 0.02 0.001-0.29	0.0059
	<i>Alu</i> ^{+/+}	16.7 (10)	26.9 (7)	0.2774	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/-}) = 0.02 0.001-0.33	0.0063
Allele	<i>Alu</i> ⁻	0.82	0.54	0.008		
	<i>Alu</i> ⁺	0.18	0.46	0.008		
		Normal group % (n)	T2DM group % (n)	<i>p</i> < 0.05	Odds ratio 95% CI	<i>p</i> value
Genotype	<i>Alu</i> ^{-/-}	80.0 (48)	36.0 (18)	0.0001	Vs (<i>Alu</i> ^{+/+} + <i>Alu</i> ^{+/-}) = 0.56 0.21-1.35	0.1843
	<i>Alu</i> ^{+/-}	3.3 (2)	36.0 (18)	0.0001	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/+}) = 0.49 0.16-1.49	0.2619
	<i>Alu</i> ^{+/+}	16.7 (10)	28.0 (14)	0.1537	Vs (<i>Alu</i> ^{-/-} + <i>Alu</i> ^{+/-}) = 0.53 0.19-1.44	0.1593
Allele	<i>Alu</i> ⁻	0.82	0.54	0.0018		
	<i>Alu</i> ⁺	0.18	0.46	0.0018		

difference in the $Alu^{-/-}$ and $Alu^{+/-}$ ($p < 0.0001$) genotype distributions between the normal non-diabetic and the DM patients. Furthermore, a significant difference was demonstrated between the Alu^{-} and Alu^{+} allelic distributions in the normal non-diabetic individuals and their DM patients' counterparts ($p < 0.0012$). On the other hand, when the diabetic patients were classified into T1DM and T2DM patients, similar genotypic and allelic distributions were noticed when they were grouped together. Table 1 shows a significant difference between normal non-diabetic individuals carrying the $Alu^{-/-}$ genotype and T1DM and T2DM individuals possessing the $Alu^{-/-}$ genotype ($p < 0.0001$). It has also been shown that the distribution of the $Alu^{+/-}$ genotype between the three tested groups exhibited the same level of significance. Comparing the genotypic distribution of the $Alu^{+/-}$ between the three experimental groups showed no significant difference. The results, assuming the recessive model (Table 1), demonstrated a significant protective effect against DM of the $Alu^{-/-}$ genotype. The $Alu^{-/-}$ genotype was 2.2 times more frequent in the normal non-diabetic population than in the diabetic population (odds ratio of 9.63, $p < 0.0001$).

DISCUSSION

The present study showed a decrease in the frequency of the $Alu^{-/-}$ genotype in the diabetic patients when compared with the normal group ($p < 0.001$; Table 1). This reduction in turn might indicate that the deletion of the Alu fragment in the tPA gene has a protective role against DM. However, the relation between the polymorphic nature of Alu insert of the tPA gene and tPA enzymatic activity or its plasma levels and thus its function is still controversial.

A number of studies have investigated the effect of circulating tPA levels on its biological activities. Works by Almer and Nilsson^[30] and Fuller *et al.*^[31] have suggested that lower tPA activity may be associated with the microthromboembolic disease. Furthermore, it has been indicated that hypofibrinolysis due to tPA levels precedes the development of T2DM in Malaysian and north Sweden subjects^[13,16,17]. Though the high levels of circulating tPA have been indicated, deficiency in tPA activity has been shown to correlate with several diseases such as cutaneous vasculitis^[32], thrombocytopenic purpura^[33], and diabetic retina^[10]. Lower tPA activity was related with an increase in the PAI-1, which binds to tPA , thus reducing tPA efficiency in converting plasminogen into plasmin and, accordingly, reducing fibrin clot lysis^[34]. Reduced fibrinolytic activity occurs in long-

term diabetic patients, which could lead to the associated microthromboembolic disease^[10]. It has long been established that T2DM is a strong cardiovascular disease risk factor^[35]. On the other hand, the $Alu^{+/-}$ genotype was associated with the elevated levels of plasma PAI-1^[12]. Nonetheless, others did not report such an association^[28,36,37].

The results of the current study demonstrated a significant protective effect against DM of the $Alu^{-/-}$ genotype. However, one shortcoming of the study was the lack of measurement of plasma levels of tPA and PAI-1, as well as assaying the activity of tPA . This limitation in turn led to our inability to associate tPA Alu specific genotype with different risk factors of DM. Nevertheless, we believe that tPA reduced activity in patients suffering from diabetes is secondary to DM and is a risk factor for blood circularity syndromes^[35]. However, further research is needed to investigate other effective genetic and environmental factors to find out the possible relationship between the tPA polymorphism and the onset of diabetes.

ACKNOWLEDGEMENTS

The authors are grateful to the Deanship of Scientific Research, The Hashemite University for supporting the current work (Grant no. 8/12/2015-7; IRB no.: 157/16/11/2015).

CONFLICT OF INTEREST. None declared.

REFERENCES

1. Sperling MA. Pediatric Endocrinology. Saunders Elsevier: USA; 2014.
2. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Canadian journal of diabetes* 2013; **37 Suppl 1**: S8-S11.
3. Piero MN, Nzaro GM, Njagi JM. Diabetes mellitus—a devastating metabolic disorder. *Asian journal of biomedical and pharmaceutical sciences* 2014; **4**(40): 1-7.
4. Schneider DJ, Sobel BE. Diabetes and thrombosis. In: Johnstone MT, Veves A (Eds.). Contemporary Cardiology: Diabetes and Cardiovascular Disease. USA: Humana Press; 2005.
5. Soares AL, Sousa MDO, Fernandes APSM, Carvalho MDG. Hemostatic changes in patients with type 2 diabetes mellitus. *Revista brasileira de hematologia e hemoterapia* 2010; **32**(6): 482-488.
6. Oszejca K, Wroński OK, Janiszewska G, Bieńkiewicz

- M, Bartkowiak J, Szemraj J. The study of *t-PA*, *u-PA* and *PAI-1* genes polymorphisms in patients with abdominal aortic aneurysm. *Molecular biology reports* 2014; **41**(5): 2859-2864.
7. Wun TC, Capuano A. Spontaneous fibrinolysis in whole human plasma. Identification of tissue activator-related protein as the major plasminogen activator causing spontaneous activity *in vitro*. *Journal of biological chemistry* 1985; **260**(8): 5061-5066.
 8. Emeis JJ. Regulation of the acute release of tissue-type plasminogen activator from endothelium by coagulation products. *Annals of the New York academy of sciences* 1992; **667**: 249-258.
 9. Parfyonova YV, Plekhanova OS, Tkachuk VA. Plasminogen activators in vascular remodeling and angiogenesis. *Biochemistry (Moscow)* 2002; **67**(1): 119-134.
 10. Luty GA, Ikedo K, Chandler C, McLeod DS. Immunolocalization of tissue plasminogen activator in the diabetic and nondiabetic retina and choroid. *Investigative ophthalmology and visual science* 1991; **32**(2): 237-245.
 11. Oliver JJ, Webb DJ, Newby DE. Stimulated tissue plasminogen activator release as a marker of endothelial function in humans. *Arteriosclerosis, thrombosis, and vascular biology* 2005; **25**(12): 2470-2479.
 12. Karadeniz M, Erdogan M, Berdeli A, Saygili F, Yilmaz C. 4G/5G Polymorphism of *PAI-1* gene and Alu-repeat I/D polymorphism of *TPA* gene in Turkish patients with polycystic syndrome. *Journal of assisted reproduction and genetics* 2007; **24**(9): 412-418.
 13. Al-Hamodi Z, Ismail IS, Saif-Ali R, Ahmed KA, Muniandy S. Association of plasminogen activator inhibitor-1 and tissue plasminogen activator with type 2 diabetes and metabolic syndrome in Malaysian subjects. *Cardiovascular diabetology* 2011; **10**: 23.
 14. Leurer C, Rabbani SA. Plasminogen Activator System—Diagnostic, Prognosis and Therapeutic Implications in Breast Cancer. In: Gunduz M eds. *A Concise Review of Molecular Pathology of Breast Cancer*. 2015. DOI: 10.5772/59429.
 15. Jern C, Ladenvall P, Wall U, Jern S. Gene polymorphism of t-PA is associated with forearm vascular release rate of t-PA. *Arteriosclerosis, thrombosis, and vascular biology* 1999; **19**(2): 454-459.
 16. Hernestål-Boman J, Norberg M, Jansson JH, Eliasson M, Eriksson JW, Lindahl B, Johansson L. Signs of dysregulated fibrinolysis precede the development of type 2 diabetes mellitus in a population-based study. *Cardiovascular diabetology* 2012; **11**: 152.
 17. Eliasson MC, Jansson JH, Lindahl B, Stegmayr B. High levels of tissue plasminogen activator (tPA) antigen precede the development of type 2 diabetes in a longitudinal population study. The Northern Sweden MONICA study. *Cardiovascular diabetology* 2003; **2**: 19-25.
 18. Yang-Feng TL, Opdenakker G, Volckaert G, Franke U. Human tissue-type plasminogen activator gene located near chromosomal breakpoint in myeloproliferative disorder. *American journal of human genetics* 1986; **39**(1): 79-87.
 19. Ludwig M, Wohn KD, Schleuing WD, Olek K. Allelic dimorphism in the human tissue-type plasminogen activator (TPA) gene as a result of an Alu insertion/deletion event. *Human genetics* 1992; **88**(4): 388-392.
 20. Tishkoff S, Ruano G, Kidd JR, Kidd KK. Distribution and frequency of a polymorphic Alu insertion at the plasminogen activator locus in humans. *Human genetics* 1996; **97**(6): 759-764.
 21. Batzer MA, Deininger PL. Alu repeats and human genomic diversity. *Nature reviews genetics* 2002; **3**(5): 370-379.
 22. Rogers J. Retroposons defined. *Nature* 1983; **301**(5900): 460.
 23. Deininger PL, Batzer MA. Evolution of Retroposons. In: Hecht M.K., MacIntyre R.J., Clegg M.T. (eds) *Evolutionary Biology*. Evolutionary Biology. USA: Springer; 1993.
 24. Batzer MA, Arcot SS, Phinney JW, Algeria-Hartman M, Kass DH, Milligan SM, Kimpton C, Gill P, Hochmeister M, Ioannou PA, Herrera RJ, Boudreau DA, Scheer WD, Keats BJB, Deininger PL, Stoneking M. Genetic variation of recent Alu insertions in human populations. *Journal of molecular evolution* 1996; **42**(1): 22-29.
 25. Stoneking M, Fontius JJ, Clifford SL, Soodyall H, Arcot SS, Saha N, Jenkins T, Tahir MA, Deininger PL, Batzer MA. Alu insertion polymorphisms and human evolution: Evidence for a larger population size in Africa. *Genome research* 1997; **7**(11): 1061-1071.
 26. Kang BY, Lee KO. Genetic polymorphisms of t-PA and PAI-1 genes in the Korean population. *Korean journal of biological sciences* 2003; **7**: 249-253.
 27. Robinson SD, Ludlam CA, Boon NA, Newby DE. Tissue plasminogen activator polymorphisms do not influence tissue plasminogen activator release in patients with coronary heart disease. *Journal of thrombosis and haemostasis* 2006; **4**(10): 2262-2269.
 28. Bahri R, Msolly A, Kassab A. Alu-repeat polymorphism in the tissue plasminogen activator gene and risks of myocardial infarction in Tunisian population. *Medicinal chemistry* 2016; **6**(2): 72-74.
 29. Hamdi HK, Reznik J, Castellon R, Atilano SR, Ong JM, Udari N, Tavis JH, Aoki AM, Nesburn AB, Boyer DS, Small KW, Brown DJ, Kenney MC. Alu DNA polymorphism in ACE gene is protective for age-related macular degeneration. *Biochemical and biophysical research communications* 2002; **295**(3): 668-672.
 30. Almer LO, Nilsson IM. On fibrinolysis in diabetes mellitus. *Acta medica scandinavica* 1975; **198**(1-2): 101-106.
 31. Fuller JH, Keen H, Jarrett RJ, Omer T, Meade TW, Chakrabarti R, North WR, Stirling Y. Haemostatic variables associated with diabetes and its complications. *British medical journal* 1979; **2**(6196): 964-966.
 32. Jordan JM, Allen NB, Pizzo SV. Defective release of tissue plasminogen activator in systemic and cutaneous vasculitis. *American journal of medicine* 1987; **82**(3): 397-400.
 33. Glas-Greenwalt P, Hall JM, Panke TW, Kang KS, Allen

- CM, Pollack VE. Fibrinolysis in health and disease: Abnormal levels of plasminogen activator, plasminogen activator inhibitor, and protein C in thrombotic thrombocytopenic purpura. *Journal of laboratory and clinical medicine* 1986; **108**(5): 415-422.
34. Grant PJ. Diabetes mellitus as a prothrombotic condition. *Journal of internal medicine* 2007; **262**(2): 157-172.
35. Mendivil CO, Robles-Osorio L, Horton ES, Hamdy O, Caballero AE. Young Hispanics at risk of type 2 diabetes display endothelial activation, subclinical inflammation and alterations of coagulation and fibrinolysis. *Diabetology and metabolic syndrome* 2003; **5**: 37.
36. Ridker PM, Baker MT, Hennekens CH, Stampfer MJ, Vaughan DE. Alu-repeat polymorphism in the gene coding for tissue-type plasminogen activator (t-PA) and risks of myocardial infarction among middle-aged men. *Arteriosclerosis, thrombosis, and vascular biology* 1997; **17**(9): 1687-1690.
37. Ladenvall P, Nilsson S, Jood K, Rosengren A, Blomstrand C, Jern C. Genetic variation at the human tissue-type plasminogen activator (tPA) locus: haplotypes and analysis of association to plasma levels of tPA. *European journal of human genetics* 2003; **11**(8): 603-610.