



TRAIL Single Nucleotide Polymorphism in Type 2 Egyptian Diabetic Patients

Ahmed Sedky Mahmoud¹, Osama Bakr Seddek²,
Shaimaa Abd EL-Kareem Mohammed¹,
Tamer Mohammed Abdel-lateef Soliman¹.

1-Department: Clinical and chemical pathology, Faculty of Medicine, Sohag university

2-Department: Clinical and chemical pathology, Faculty of Medicine, Assuit University

Abstract

Objective: The research attempted to assess the susceptibility of TRAIL polymorphism (rs1131580) in T2DM and its relationship with metabolic parameters in T2DM.

Patients and Methods: This study was performed on 80 subjects, 60 T2DM patients and age & sex matched 20 healthy volunteers as controls, The patients had complete clinical examination and detailed history collection, and detection of TRAIL single nucleotide polymorphism rs1131580 by PCR-RFLP standing for polymerase chain reaction-restriction fragment length polymorphism..

Results: TRAIL SNP rs1131580 mutation was greatly more common in the group of diabetics.

Conclusion: A homozygous variant allele (CC) genotype of the TRAIL SNP rs1131580 carries a 1.5-fold risk of DM. Also, having the C allele elevated the risk of T2DM by 1.7 times.

Keywords: Tumor necrosis factor-related apoptosis-inducing ligand, PCR-RFLP, T2DM.

DOI : 10.21608/SMJ.2023.226952.1402

*Correspondence : sakma201016@gmail.com

Received: 05 August, 2023.

Revised: 23 August, 2023.

Accepted: 23 August, 2023.

Published: 30 August, 2023.

Introduction

Diabetes mellitus (DM) is a group of metabolic illnesses which results in high blood glucose level caused by inadequate insulin production by pancreatic beta-cells.⁽¹⁾ The incidence and prevalence of DM have considerably increased everywhere in the world. According to the most current data, the prevalence of DM has increased to 9.1% globally and 11.7% in China. 642 million individuals worldwide will have diabetes by 2040.⁽²⁾ Obesity, hypertension, high cholesterol, and the metabolic syndrome all raise the risk of developing type 2 diabetes mellitus (T2DM). Strong heritable genetic elements are also found in T2DM.⁽³⁾ T2DM may result from the abnormal expression of a variety of genes, including tumor necrosis factor (TNF), Interleukin -1 and tumor growth factor.⁽⁴⁾ Apo-2L, commonly known as TRAIL or TNF-related apoptosis-inducing ligand,

is a brand-new member of the TNF family. In 1995, Wiley discovered TRAIL for the first time.⁽⁵⁾ A multifunctional cytokine called TRAIL has strong anti-tumor effects, most likely via inducing cell death. Additionally, TRAIL modulates the pathophysiological processes of autoimmune, metabolic, and cardiovascular illnesses.⁽⁶⁾ The genesis of metabolic diseases as obesity, diabetes, and hypercholesterolemia may be significantly influenced by TRAIL. In apolipoprotein E-deficient mice, an element deletion of the TRAIL gene increased body weight, hyperglycemia, insulin resistance, and high cholesterol.⁽⁷⁾ Both in T1DM and in T2DM, the idea of TRAIL as a new participant in the etiology of diabetes has recently been demonstrated.⁽⁸⁾ As a naturally occurring vascular tone regulator, TRAIL may contribute to vascular abnormalities in diabetes by tending to

decrease in serum levels in T2DM patients after insulin treatment.⁽⁸⁾ The relationship between TRAIL (TNFSF10) single nucleotide polymorphisms and T2DM has not been established. At nucleotide 1595, TRAIL's TT genotype was shown to be associated with a lower prevalence of fatty liver disease, which is tied to the onset and progression of T2DM.⁽⁹⁾

Subjects and Methods

This research was performed on 80 subjects, 60 T2DM patients attending diabetic clinic in Sohag University Hospital in the period from December 2020 to August 2021, and age and sex-matched healthy participants as controls. They had been divided into two main groups: a T2DM group which included 60 patients with T2DM and a control group which included 20 apparently healthy individuals of both sexes.

1. **Ethical considerations:** Sohag University, Faculty of Medicine's Scientific and Ethical Committee made revisions to this research. Written informed consent was acquired from the patients and controls after discussing with them the aim of the study and methods.
2. **Inclusion criteria:** Patients with T2DM according to American Diabetes Association and World Health Organization standards, Egyptian patients older than 30 years old.
3. **Sample collection:**
 - a. Eight ml venous blood samples were taken from all patients and healthy volunteers under aseptic conditions. Samples taken were divided into 3 portions: two ml of blood on Ethylene diamine tetra acetic acid (EDTA) for genomic study using PCR – RFLP technique, two ml of blood on EDTA for glycated hemoglobin (HbA1c) and complete blood count, and four ml of blood on the plain tube and left to clot then centrifugation and separation of the serum in aliquot for blood chemistry.
 - b. Urine sample was taken from patients and controls for urine analysis and albumin creatinine ratio.
4. **Laboratory investigations:** investigations included are:
 - a. **Routine investigations:** HbA1C, fasting blood glucose, fasting insulin, HOMA IR, kidney function test, complete blood count, liver function test, lipid profile, urine analysis, and albumin creatinine ratio.

b. Detection of TRAIL single nucleotide polymorphism (rs1131580) using PCR-RFLP technique:

i. DNA Extraction:

Using the Gene JET whole Blood Genomic DNA Purification Mini Kit#K0781,#K0782 from Thermo Scientific (USA). For SNP analysis, the acquired genomic DNA was kept in a freezer at - 20°C.

ii. TRAIL genotyping:

SNP genotyping was carried out using PCR-RFLP, or polymerase chain reaction-restriction fragment length polymorphism. Based on TRAIL gene sequence and a prior work, specific primers were created.⁽¹⁰⁾ 100 ng DNA template, 0.5 mL dNTPs, 2.5 mL PCR buffer, 10 pM forward primer (5-AACATCTTCTGTCTTT-ATAATC-3), 10 pm reverse primer (5-AAATAACACGTACTIONACTGAAG-3), and 1.25 μTaq-DNA-polymerase were employed to perform the PCR. The PCR cycling settings were 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec, 48°C for 45 sec, and 72°C, with a final extension step at 72°C for 5 min. Thermo scientific TasI restriction enzyme was used to digest PCR products for 3 hours at 65°C. The dissolved components were examined by 2.5% agarose gels electrophoresis and were made visible with ethidium bromide staining. DNA sequencing of 2 randomly chosen samples verified the desired TRAIL genotypes.

5. **Statistical analysis:** IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA) was used for the statistical analysis. The right way to express numerical data was using the mean, standard deviation, or median and range. Frequency and percentage were used to convey qualitative data. The relationship between qualitative variables was investigated using the Fisher's exact test or Pearson's Chi-square test. When comparing two sets of numerical data, either the Student t-test or the Mann-Whitney test (a non-parametric t-test) were used, depending on whether the data were regularly distributed or not.

Utilizing a non-parametric ANOVA, the Kruskal-Wallis test was used to compare the two groups. After adjusting for age and triglyceride as factors, risk was calculated using

logistic regression analysis. For the purpose of estimating risk, an odds ratio (OR) with a 95% confidence interval (CI) was utilized. There were two tails on each exam. A 0.05 p-value was regarded as significant.

Results:

1. Demographic data of patients and control groups

In this study, the average age of the 60 diabetes patients was 51.88 ± 8.03 years (31-71 years) for 28 females and 32 men, whereas the aver-

age age of the 20 controls was 41.60 ± 12.93 years (20-71 years) for 12 females and 8 males.

2. rs1131580 SNP in TRAIL was genotyped:

At position 1595 in TRAIL, 3 genotypes were found in all the enrolled participants. The PCR products were divided into 291- and 193-bp fragments (for the genotype CC), 291-, 193-, 131-, and 62-bp fragments (for the genotype CT), or 291-, 131-, and 62-bp fragments (for the genotype TT) after being digested with *TasI*.(Figure1)

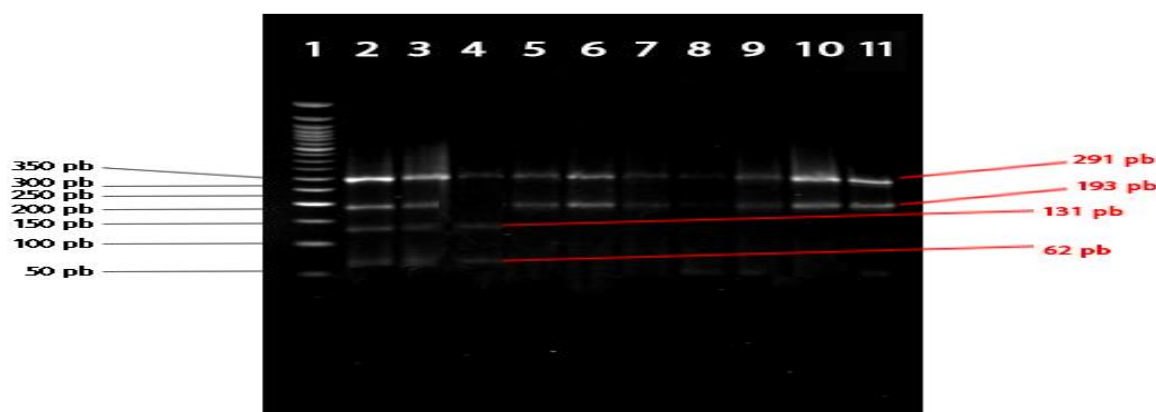


Figure (1): Agarose gel electrophoresis for (rs1131580) after digestion with *TasI* enzyme in diabetic patients. Lane (1): 50 bp DNA marker, Lanes 2,3: (CT) genotype with four fragments 291, 193, 131, and 62 bp. Lane 4 (TT) genotype with three fragments 291, 131, and 62 bp. Lanes 5,6,7,8,9,10,11: (CC) genotype with two fragments 291 and 193 bp.

3. rs1131580 genotype and allele frequencies in the two groups under investigation:

The prevalence of the wild homozygous genotype carriers (TT) in whole patients and controls was 16.7% and 30% respectively. The overall prevalence of (rs1131580) (CT and CC) mutant genotypes in whole diabetic patients was 83.3%(50% heterozygous and 33.3% homozygous) while in controls was 70% (45% heterozygous and 25% homozygous).So, TRAIL SNP rs1131580 mutation was more

common in diabetic group when compared to the control group with high significance ($P < 0.0001$).

In the T2DM group compared to the control group, the frequency of the C allele of SNP rs1131580 was significantly higher (T2DM group vs control group: 61.1% vs 47.5%, $p < 0.05$, OR = 1.737, 95% CI = 0.819 - 3.682) as shown in table1.

Table (1): TRAIL rs1131580 genotype and allele frequencies in the two studied group.

Group	n	Genotype frequency ^a , n (%)			P value	Allele frequency ^b , n (%)		P value
		CC	CT	TT		C	T	
T2DM	60	20 (33.3%)	30 (50%)	10 (16.7%)	<0.0001	55 (66.1%)	35(38.9%)	<0.05
Control	20	5 (25%)	9 (45%)	6 (30%)		19 (47.5%)	21(52.2%)	

^a T2DM group vs control group, $P < 0.0001$; ^b T2DM group vs control group, $P < 0.05$, OR = 1.737, 95% CI = 0.819-3.68

Using logistic regression of the SNP rs1131580 model, the homozygous CC genotype increases the risk of T2DM by 1.5 times, with an OR of 1.500 (95% CI: 0.477 – 4.717) as shown in(table 2).

Table (2): Risk estimation of DM with SNP rs1131580 genotype.

	P value	Odds ratio	95% CI for the Odds ratio	
			Lower	Upper
CT vs. TT	<0.05	1.222	0.443	3.376
CC vs. TT	<0.05	1.500	0.477	4.717

Table (3): Comparison between the routine laboratory tests of the two studied groups.

Parameters	Unit	T2DM (mean ± SD)	Control (mean ± SD)	P value
Fasting Blood Glucose	mg/dL	244.1 ± 100.25	84.20 ± 9.84	<0.0001
Fasting Insulin	µIU/mL	23.3 ± 14.58	13.5 ± 5.33	0.003
HOMA IR		2.9 ± 1.76	1.1 ± 0.509	<0.0001
HbA1c	%	9.13 ± 2.01	4.63 ± 0.33	<0.0001
Cholesterol	mg/dL	182.5 ± 52.08	174.05 ± 43.21	0.516
Triglycerides	mg/dL	206.25 ± 109.5	143.1 ± 34.3	0.013
Very Low-Density Lipoprotein	mg/dL	40.5 ± 21.8	25.2 ± 3.97	0.003
Low-Density Lipoprotein	mg/dL	106 ± 20	90.2 ± 19.3	0.005
High-Density Lipoprotein	mg/dL	40.4 ± 10.12	44.3 ± 4.77	0.024
Alanine Transferase	U/L	22.3 ± 15.96	16.0 ± 4.87	0.008
Aspartate Transferase	U/L	22.28 ± 16.92	16.75 ± 6.22	0.036
Total Protein	g/dL	7.82 ± 0.58	8.07 ± 0.26	0.012
Albumin	g/dL	4.05 ± 0.51	4.15 ± 0.27	0.388
Total Bilirubin	mg/dL	0.53 ± 0.32	0.54 ± 0.21	0.881
Direct Bilirubin	mg/dL	0.14 ± 0.13	0.085 ± 0.039	0.003
Creatinine	mg/dL	0.92 ± 0.95	0.59 ± 0.26	0.021
Urea	mg/dL	29.16 ± 30.04	21.10 ± 12.78	0.098
Albumin Creatinine Ratio	mg/g	146.8 ± 88.9	11.6 ± 4.4	<0.001
White Blood Cells Count	×10 ³ /µl	8.71 ± 3.81	7.32 ± 2.02	0.043
Red Blood Cells Count	×10 ⁶ /µl	4.76 ± 0.63	4.52 ± 0.51	0.133
Hemoglobin	g/dL	12.39 ± 1.54	12.28 ± 1.09	0.759
Platelet count	×10 ³ /µl	259.57 ± 74.79	268.1 ± 81.93	0.667

3. Correlation between the routine laboratory tests in T2DM patient with different TRAIL genotypes:

At site 1595 in TRAIL, patients were divided into 3 groups based on their genotypes (CC, CT and TT), and the results of the routine laboratory tests in these groups were evaluated in Table 4.

Table (4): Correlation between the routine laboratory tests in patients according to various genotypes of TRAIL.

Parameters	Unit	CC (mean ± SD)	CT (mean ± SD)	TT (mean ± SD)	P value
Fasting Blood Glucose	mg/dL	223.4 ± 88.46	269.77 ± 103.92	200.0 ± 91.28	0.002
Fasting Insulin	µIU/mL	23.75 ± 18.16	22.47 ± 11.34	25 ± 16.66	0.029
HOMA IR		2.75 ± 1.83	3.01 ± 1.62	3.05 ± 2.17	0.012
HbA1c	%	9.06 ± 2.17	9.37 ± 1.89	8.41 ± 2.01	0.022
Cholesterol	mg/dL	178.75 ± 61.04	183.90 ± 48.88	183.30 ± 44.34	0.613
Triglycerides	mg/dL	197.15 ± 117.27	218.52 ± 115.36	181.00 ± 64.43	0.189
Very Low-Density Lipoprotein	mg/dL	39.19 ± 23.40	42.50 ± 23.04	35.82 ± 12.77	0.242
Low-Density Lipoprotein	mg/dL	40.11 ± 21.1	38.30 ± 19.27	44.61 ± 23.61	0.324
High-Density Lipoprotein	mg/dL	40.30 ± 11.15	39.19 ± 9.54	45.80 ± 9.60	0.023
Alanine Transferase	U/L	23.90 ± 25.05	20.81 ± 8.19	23.80 ± 10.28	0.658
Aspartate Transferase	U/L	24.10 ± 26.23	19.71 ± 6.69	26.00 ± 15.33	0.250
Total Protein	g/dL	7.85 ± 0.64	7.88 ± 0.58	7.6 ± 0.45	0.106
Albumin	g/dL	3.94 ± 0.45	4.09 ± 0.51	4.09 ± 0.61	0.099
Total Bilirubin	mg/dL	0.50 ± 0.29	0.50 ± 0.28	0.71 ± 0.43	0.002
Direct Bilirubin	mg/dL	0.14 ± 0.11	0.14 ± 0.11	0.17 ± 0.21	0.025
Creatinine	mg/dL	1.03 ± 1.18	0.85 ± 0.87	0.88 ± 0.74	0.600
Urea	mg/dL	26.90 ± 25.43	27.97 ± 26.76	35.70 ± 46.16	0.472
Albumin Creatinine Ratio	mg/g	36.8 ± 85.21	21.87 ± 56.3	54.4 ± 139.8	0.032
White Blood Cells Count	×10 ³ /µl	8.17 ± 2.79	8.39 ± 2.94	10.27 ± 6.98	0.108
Red Blood Cells Count	×10 ⁶ /µl	4.65 ± 0.71	4.75 ± 0.59	4.92 ± 0.67	0.050
Hemoglobin	g/dL	11.83 ± 1.60	12.50 ± 1.56	13.02 ± 1.06	<0.0001
Platelet count	×10 ³ /µl	283.00 ± 95.08	258.39 ± 64.63	207.40 ± 17.49	<0.0001

Discussion

DM is a series of metabolic disease characterized by high blood glucose level brought on by decreased insulin secretion, insulin function, or both.⁽¹¹⁾ Type 2 diabetes is brought on by a confluence of environmental and genetic risk factors. Environmental risk factors include sedentary lifestyles such a high incidence of excessive calorie consumption, decreased physical exercise, smoking, and heavy alcohol usage. Environmental risk factors also include internal environmental indicators like inflammation, adipocytokines, and hepatocyte factors as well as exterior environmental variables like environmental endocrine disruptors.⁽¹²⁾ TNF superfamily member TRAIL induces cell death.⁽¹³⁾ There has been an increase in interest in using TRAIL's potential to treat these diseases as a result of an increasing number of experimental and clinical evidence suggesting that the TRAIL system plays a significant role in the onset and progression of both autoimmune (T1DM) and obesity-related metabolic diseases (T2DM).⁽¹⁴⁾

As regards TRAIL SNP rs1131580 mutation, it was observed that the prevalence of the wild genotype carriers (TT) was 16.7% in the patients and 30% in controls. The percentage of mutant genotypes of (CT, and CC) in whole diabetic patients was (50% heterozygous and 33.3% homozygous) while in controls were (45% heterozygous and 25% homozygous).

The distribution of (rs1131580) mutant genotypes viewed a highly significant increase in whole patients when compared to controls ($p < 0.0001$). The frequency of mutant C allele in whole patients and controls was 61.1% and 47.5% respectively which was obviously greater in whole patients when compared to controls ($p < 0.05$).

This distribution was close to a study done by Yu et al. in 2013 in which T2DM participants compared to healthy controls and the frequency of the CC genotype was considerably greater (T2DM group vs. control group: 32.19 vs. 25.94%, $P < 0.05$).⁽¹⁵⁾ Furthermore, there was an important distinction between the T2DM group and the control group in the frequency of the C allele of SNP rs1131580 (T2DM group vs control group: 56.85 vs 48.87%, $P < 0.01$).

Comparing whole patients to controls revealed that mutation of (rs1131580) gene(CC genotypes) increased the risk of T2DM 1.5 times (OR=1.500, 95% CI= 0.477 – 4.717, $p < 0.05$) and the C allele

elevated the risk of T2DM by 1.73 times compared to controls. (OR=1.737, 95%CI = 0.819 – 3.682, $p < 0.05$). This was in agreement with a study done by Yan et al. who reported that the C allele increased the incidence of T2DM by 1.3 times (OR = 1.378, 95% CI = 1.088 - 1.745, $P < 0.01$).⁽¹⁶⁾

There was a strong correlation between the presence of (rs1131580) mutant genotypes and fasting blood glucose ($p=0.002$) also there was a significant association with HbA1C ($p=0.022$). This was consistent with the findings of the study by Yu et al. who said that fasting blood glucose levels in the T2DM group were considerably higher than those in the control group.⁽¹⁵⁾

In our study, significant correlation was discovered between mutant genotypes (CC and CT) of (rs1131580) with HDL ($p=0.23$), however, there was no significant correlation was observed between mutant genotypes of (rs1131580) with cholesterol, triglyceride, and VLDL. Yu et al. found that levels of TC, HDL, and LDL in T2DM patients with various TRAIL genotypes did not differ significantly from one another, which is in agreement with our study. Conversely to our findings, the amount of TG in T2DM patients with genotype CC was considerably greater than that in T2DM patients with genotype TT.⁽¹⁵⁾

In our study, there was significant association was observed between mutant genotypes of (rs1131580) with fasting insulin, HOMA IR, and albumin creatinine ratio ($p= 0.029, 0.012, \text{ and } 0.032$ respectively). These are new parameters in our study, and it is difficult to find similar results in other papers to compare with them.

Conclusion

A 1.5-fold higher risk of T2DM is linked to the homozygous variant allele (CC) genotype of the TRAIL SNP rs1131580. In addition, the C allele seems to make people 1.7 times more likely to develop T2DM than people without the mutation.

References

1. **World Health Organization.** Global Report on Diabetes, 2016; 88.
2. **International Diabetes Federation.** IDF Diabetes Atlas, 2015; 7th ed.

3. **Freeman H and Cox RD.** Type-2 diabetes: a cocktail of genetic discovery. *Hum. Mol. Genet.*, 2006; 15 Spec No 2: R202-R209.
4. **Lin HM, Lee JH, Yadav H, Kamaraju AK, et al.** Transforming growth factor-beta/Smad3 signaling regulates insulin gene transcription and pancreatic islet beta-cell function. *J. Biol. Chem.*, 2009; 284: 12246-57.
5. **Wiley SR, Schooley K, Smolak PJ, Din WS, et al.** Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 1995; 3: 673-82.
6. **Neve A, Corrado A and Cantatore FP.** TNF-related apoptosis-inducing ligand (TRAIL) in rheumatoid arthritis: what's new? *Clin Exp Med.*, 2014; 14: 115–20.
7. **Di Bartolo BA, Chan J, Bennett MR, Cartland S, Bao S, et al.** TNF-related apoptosis-inducing ligand (TRAIL) protects against diabetes and atherosclerosis in Apoe (-)/(-) mice. *Diabetologia.*, 2011; 54: 3157–67.
8. **Koliaki C. &Katsilambros N.** Repositioning the Role of Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) on the TRAIL to the Development of Diabetes Mellitus: An Update of Experimental and Clinical Evidence. *International Journal of Molecular Sciences*, 2022; 23(6), 3225.
9. **Li Y, He S, Li C, Shen K, Yang M, Tao W, Yang Y, Shi L, Yao Y.** Evidence of association between single-nucleotide polymorphisms in lipid metabolism-related genes and type 2 diabetes mellitus in a Chinese population. *Int J Med Sci.* 2021; 1;18(2):356-63.
10. **Gray H.L., Sorensen E.L., Hunt J.S. and Ober C.** Three polymorphisms in the 3' UTR of the TRAIL (TNF-related apoptosis-inducing ligand) gene. *Genes Immun.* 2001; 2: 469-70.
11. **Padhi S., Amit Kumar Nayak and Anindita Behera.** Type II diabetes mellitus: a review on recent drug-based therapeutics. *Biomedicine & Pharmacotherapy*, 2020; 131: 708-19.
12. **Bi Y., Wang T., Xu M., Xu Y., Li M., Lu J. & Ning G.** Advanced research on risk factors of type 2 diabetes. *Diabetes/metabolism research and reviews*, 2012; 28, 32-39.
13. **Chan J., Prado-Lourenco L., Khachigian L.M., Bennett M.R., et al.** TRAIL promotes VSMC proliferation and neo intima formation in an FGF-2-, Sp1 phosphorylation-, and NF kappa B-dependent manner. *Circ Res*, 2010; 106:1061–71.
14. **Koliaki C. & Katsilambros N.** Repositioning the Role of Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) on the TRAIL to the Development of Diabetes Mellitus: An Update of Experimental and Clinical Evidence. *International Journal of Molecular Sciences*, 2022; 23(6), 3225.
15. **Yu M.Y., Zhao P.Q., Yan X.H., Liu B., X.H., Zhu F.L. and Gao L.F.** TRAIL single nucleotide polymorphism and type 2 diabetes. *Genetics and Molecular Research*, 2013; 12 (3): 3455-64.
16. **Yan X.H., Liu B., Zhang Q.Q., Wang R., Ma C.H., Liang X.H., Zhu F.L. and Gao L.F.** TRAIL single nucleotide polymorphism and type 2 diabetes. *Genetics and Molecular Research*, 2013; 12 (3): 3455-64.