Association of IGF-I gene polymorphism with diabetic nephropathy in Egyptians with type 2 diabetes

Mohamed A. M. Hegazi[a], Amal Seleem[b], Eman H. EL-Adawy[c], Mahmoud E. A. Elhussini[a]

[a]Department of Physiology and Zoology, Faculty of Science, Tanta University, Tanta, [b]Department of Biochemistry, [c]Department of Internal Medicine, Endocrinology Unit, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Correspondence to Eman H. El-Adawy, MD, Department of Internal Medicine, Endocrinology Unit, Faculty of Medicine, Specialized Medical Hospital, Mansoura University, Mansoura, 2554, Egypt.
Tel; 0502230176; fax: 0502230176;
e-mail: emaneladawy@yahoo.com

Received 2 July 2018
Accepted 6 August 2018

The Egyptian Journal of Internal Medicine
2018, 30:191–196

Background
Genetic-based studies are being carried out worldwide to identify the susceptibility genes for diabetic nephropathy (DN), which represents a major health problem in all diabetics.
Aim
To study the association between genotypes of insulin-like growth factor-1 (IGF-1) and DN among Egyptian patients with type 2 diabetes and if there is any possible relation between these polymorphisms and different variables.
Patients and methods
A total of 26 diabetics without nephropathy (group 1) and 26 with nephropathy (group 2), with an average age of 52.7±6.1 years, and 25 age-matched and sex-matched healthy participants (control group) were included. Two tagging single nucleotide polymorphisms were assessed in IGF-1 gene: rs6214 and rs10860860. Genotypic distribution was tested for Hardy–Weinberg equilibrium. The genotype was evaluated using the χ^2 tests. Fasting blood glucose, glycated hemoglobin, uric acid, lipid profile, serum creatinine, and urine albumin–creatinine ratio were assessed.
Results
The distribution of IGF-1 gene polymorphisms reflects a significant association with DN, where the frequency of variant genotype GG in polymorphism rs6214 was found to be significantly higher in diabetics with nephropathy than other groups [odds ratio (OR)=20.57; 95% confidence interval, 2.25–74; *P*=0.001). Moreover, the frequency of variant AA in polymorphism rs10860860 was also found to be significantly higher in diabetics with nephropathy (OR=7.37; 95% confidence interval, 1.87–30.07; *P*=0.001). However, GA and AT alleles were found to be associated with ORs less than 1 in diabetics with nephropathy when compared with other groups (*P*=0.002 and 0.007, respectively), which means that it could be significantly protective against DN. where they found to be significantly higher in diabetics with genotype GG than those with genotype GA (*P*=0.13, 0.11 respectively).
Conclusion
The variants of IGF-1 rs6214 and rs10860860 could entail a risk of DN in Egyptians with type 2 diabetes mellitus. It means that the type of IGF-1 gene polymorphism is responsible for the susceptibility of DN more than its serum level.

Keywords:
diabetic nephropathy, insulin-like growth factor-1 polymorphism, type 2 diabetes mellitus

Egypt J Intern Med 30:191–196

1110-7782

Introduction

Generally speaking, growth factors are believed to play a crucial role in the development of diabetic vascular complications, and the functional effect of these factors is usually related to the genetic distribution rather than its level [1,2].

It has been shown that insulin-like growth factor-1 (IGF-1) has been linked to the late microvascular diabetes complications, including diabetic nephropathy (DN) [3,4]. The circulating IGF-1 level may not adequately reflect the IGF-1 bioactivity, especially in pathological conditions, owing to the interference of IGF-1-binding proteins, so an alternative approach may be genetic-based studies [5].

IGF-1 gene is located on the long arm of chromosome 12q22-24.1, and the encoded IGF-1 protein is a single chain polypeptide with 70 amino acids [6]. Both IGF-1 and its specific receptor (IGF-IR) have strong structural homology with pro-insulin and insulin

receptor. Consequently, they exert significant effects on glucose and protein metabolism in addition to their mitogenic effects, thus contributing to the main pathways in the exacerbation and/or development of diabetic microangiopathic complications [7,8].

Given that DN is the leading cause of end-stage renal disease [9] and that IGF-1 has an established role in the diabetic microangiopathic triad, we hypothesized that two single nucleotide polymorphisms (SNPs) in IGF-1 gene may be associated with risk predisposition to DN, so we investigate the risk allele frequency and its association with DN.

Patients and methods

This case–control study included 52 patients with type 2 diabetes (11 male and 14 female), with mean age of 52.7±6.1 year, selected from the Outpatient Clinics of Mansoura University Hospital, Faculty of Medicine, Egypt, from February 2016 to March 2017. Moreover, 25 healthy controls matched for sex and age were also selected from the same locality. Informed consents were obtained from all participants, and approval was given by the ethics committee of our institution.

Full history taking and clinical examination were taken from all participants. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood pressure was taken in the sitting position using a random-zero sphygmomanometer. Exclusion criteria included presence of nondiabetic renal disorders (i.e. abnormal kidney on renal ultrasound, surgical nephrectomy or solitary kidney, urologic disorders, and suspicion of other nondiabetic renal diseases). We selected 26 patients with nephropathy and 26 without nephropathy, where nephropathy was defined as microalbuminuria [albumin-to-creatinine ratio (ACR) >3.5 in men and >2.5 in women], macroalbuminuria (ACR>30), and/or renal impairment (creatinine clearance ≤60 ml/min plus serum creatinine >1.4 mg/dl in men or >1.2 mg/dl in women) [10].

Venous blood samples were collected after an overnight fasting. Serum triglyceride level was estimated according to the method of Allain *et al.* [11]. Serum total cholesterol (TC) was estimated according to the method of Fossati and Prencipe [12]. Serum high-density lipoprotein-cholesterol (HDL-C) was estimated according to the method of Williams *et al.* [13]. Low-density lipoprotein-cholesterol (LDL-C) level in blood serum was calculated according to the following equation: LDL-C=TC–HDL-C–triglyceride/5, which was described by Trinder [14]. Estimation of blood glucose was performed according to the method of Trivedi *et al.* [15]. Uric acid level in blood serum was determined according to the method of Vasiliades [16]. Creatinine level in blood serum was determined according to the method of Talke and Schubert [17]. All kits were provided by Elitech diagnostics (Zone Industrielle, Sees, France). Glycated hemoglobin was determined using a kit purchased from biotech kit (Biotech, Cairo, Egypt) [18].

DNA extraction

Genomic DNA was isolated from EDTA anticoagulated peripheral blood using a DNA extraction kit (Qia-ampilification extraction kit; Qiagen, New York, Frankfurt, USA).

Principle of DNA extraction

DNA extraction is the process by which DNA is separated from proteins, membranes, and other cellular material contained in the cell from which it is recovered. Samples are digested with proteinase K, which is the protease used to digest proteins from samples. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane; impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the elution buffer. The purity of template DNA is detected by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. An A260/A280 ratio of 1.7–1.9 means pure template DNA and is better suited for PCR [19]

Procedure

The procedure was as follows:

(1) 200 µl of EDTA anticoagulated blood sample was used for DNA extraction according to the manufacturer's instructions.
(2) The extracted DNA was verified by agarose gel electrophoresis (1% ethidium bromide stained agarose).
(3) The concentration and purity were tested spectrophotometrically by measuring absorbance at 260/280 nm.
(4) The extracted DNA samples were stored at −80°C until amplification by PCR.

Amplification

PCR:

(1) Amplification of IGF-1 gene using the PCR: enzymatic amplification was performed by PCR using Taq polymerase enzyme and PTC-100 thermal cycler (MJ Research Inc., Watertown,

Massachusetts, USA). Regarding primer sequences, we used the following oligonucleotide primers for in-vitro amplification of specific IGF-1 gene fragment:
Amplification of IGF-1 (rs6214) SNP. The following primers were used:
5′-GATGGCACTTCTTTTTATTTCTTG-3′ (primer 5′ or forward) and 5′-TGGCAGT GCATCTTTCAGA-3′ (primer 3′ or reverse).
Amplification of IGF-1 (rs10860860) SNP. The following primers were used: 5′-AGGACCTG GCAAATGATG-3′ (primer 5′ or forward) and 5′-TATGGCAATTACATATTGGAATG 3′ (primer 3′ or reverse).

(2) IGF-1 gene polymorphism:
The total volume of 25 ml included 30 ng genomic DNA, 0.3 mmol of each primer (Promega, Madison, Wisconsin, USA), and 1× PCR mix (Taq PCR Master Mix Kit; Qiagen GmbH, Hilden, Germany) containing 200 mmol/l of each dNTP, 5 ml 10× reaction buffer, 1.25 U Taq Gold Polymerase, and 4 mmol/l $MgCl_2$.

Cycling condition

For IGF-1 (rs6214) SNP determination, a thermal cycler was used as follows: denaturation at 94°C for 5 min, followed by 35 cycles under the following conditions:

(a) Denaturation at 94°C for 1 min.
(b) Annealing at 60°C for 40 s.
(c) Extension at 72°C for 1 min.
(d) Final extension cycle at 72°C for 5 min.

PCR product size (214 bp) was digested by restriction enzyme, TaiI, according to kit instructions (New England Biolabs, Beverly, Massachusetts, USA) at 65°C for 16 h. Electrophoresis of 5 µl of the PCR product was separated on 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

For IGF-1 (rs10860860) SNP determination, a thermal cycler was used as follows: denaturation at 94°C for 5 min, followed by 35 cycles under the following conditions:

(1) Denaturation at 94°C for 1 min.
(2) Annealing at 58°C for 40 s.
(3) Extension at 72°C for 1 min.
(4) Final extension cycle at 72°C for 5 min.

PCR product size (417 bp) was digested by restriction enzyme, NdeI, according to kit instructions (New England Biolabs) at 37°C for 16 h. Electrophoresis of 5 µl of the PCR product was separated on 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Results

Table 1 showed insignificant difference between groups regarding age and sex (P=0.08 and 0.5, respectively). However, the mean value of BMI was significantly higher among group with nephropathy when compared with those without nephropathy (P=0.01). The mean values of lipid profile, TC, triglyceride, and LDL-C were statistically higher in diabetics with nephropathy than those without nephropathy, whereas HDL-C was significantly lower in nephropathy. The other tested parameters such as fasting blood glucose, glycated hemoglobin, serum creatinine, uric acid, and ACR also showed statistical significant higher values in diabetics with nephropathy than diabetics without nephropathy.

Tables 2 and 3 showed the frequency distribution of genotypes of IGF-1 SNPs rs6214 and rs10860860 among the study groups. They revealed that GA and AT alleles were associated with odds ratios (ORs) less than 1 in diabetics with nephropathy when compared with other groups (P=0.002 and 0.007, respectively), which means that they could be significantly protective against DN. However, GG and AA alleles showed ORs more than one among diabetics with nephropathy, therefore they could carry the risk predisposition for DN (P=0.001 for both).

The associations between genotype and laboratory parameters related to DN were also investigated (Tables 4 and 5). In genotypes of SNP rs6214, we found the mean values of LDL and serum creatinine showed genotype-dependent variations convenient with poorer renal function, where they were found to be significantly higher in diabetics with genotype GG than those with genotype GA (P=0.13 and 0.11, respectively). However, other parameters did not show any significant differences among the three alleles. However, in the genotypes of IGF-1 SNP rs10860860, we found the mean values of TC, LDL, and uric acid were significantly higher in diabetics with genotype AA than those with genotype AT (P=0.10, 0.25, and 0.37, respectively). However, other parameters did not show significant differences.

Discussions

Among the triad of microangiopathic diabetic complications, only DN can lead to death [9].

Table 1 Clinical and biochemical characteristics of the study groups

Variables	Control (group 1) (*N*=25)	DM without nephropathy (group2) (*N*=26)	DM with nephropathy (group3) (*N*=25)	*P*
Age (mean±SD) (year)	52.7±6.1	53.95±9.01	56.93±10.53	0.08
BMI (mean±SD)	27.70±3.45	31.25±5.57	33.53[a]±7.41	0.01
Sex [*n* (%)]				
Male	11 (44.0)	12 (46.1)	10 (38.5)	0.5
Female	14 (56.0)	14 (53.8)	16 (61.5)	
Serum cholesterol (mg/dl)				
Mean±SD	154.4±15.51	216.56±25.46[a]	276.56±24.95 [b,c]	<0.001*
Range	128–193	169–281	231–361	
Serum triglycerides (mg/dl)				
Mean±SD	80.2±16.27	167.84±9.33[a]	209.56±25.34[b,c]	<0.001*
Range	43–101	147–183	156–271	
HDL-C (mg/dl)				
Mean±SD	64.28±9.98	60.30±8.49	39.96±7.24[b,c]	<0.001*
Range	42–79	43–83	28–59	
LDL (mg/dl)				
Mean±SD	74.47±17.21	122.17±25.95[a]	194.68±27.82[b,c]	<0.001*
Range	47.2–105.2	79–164.1	143.4–283.4	
Serum creatinine (mg/dl)				
Mean±SD	0.84±0.11	1.14±0.25	3.2±2.07[b,c]	<0.001*
Range	0.61–1.05	0.71–1.55	0.87–11	
Serum glucose fasting (mg/dl)				
Mean±SD	82.72±7.35	245.89±41.14[a]	261.659±59.7187[c]	<0.001*
Range	73–97	178–305	100–378	
HbA1c (%)				
Mean±SD	5.38±0.631	7.9 a±0.85	9.14±1.62[b,c]	<0.001*
Range	4.3–6.2	6.80–8.5	6.3–11.7	
Serum uric acid (mg/dl)				
Mean±SD	4.19±0.596	4.53±0.917	6.84±1.75[b,c]	<0.001*
Range	3–5.6	2.3–6.1	4.3–11.5	
ACR (mean±SD) (mg/g)	14.26±7.05	14.27±9.4	1263.64±1502.2[b,c]	<0.001*

One way analysis of variance test was used. ACR, albumin–creatinine ratio; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein. [a]Significance between patients with DM without nephropathy and control. [b]Significance between patients with DM with nephropathy and patients with DM without nephropathy. [c]Significance between patients with DM with nephropathy and control. Significance was considered at *P* value less than 0.05. *Means statistically significant.

Table 2 Frequency distribution of genotypes of insulin-like growth factor-1 single nucleotide polymorphism rs6214 among the study groups

rs6214 (%)	Control [*n* (%)]	DM without nephropathy [*n* (%)]	DM with nephropathy [*n* (%)]	OR_1	OR_2	P_1	P_2
AA	8 (32)	7 (26.9)	7 (26.9)	0.8 (.20–3.1)	0.98 (.25–3.8)	0.75	0.97
GA	6 (64)	15 (57.7)	7 (26.9)*	0.8 (21–2.7)	0.21 (.05–0.8)	0.74	0.002*
GG	1 (4)	4 (15.4)	12 (46.2)*	4.36 (0.4–7)	20.57 (2.3–74)	0.091	0.001*

Test used, χ^2. DM, diabetes mellitus; OR, odds ratio. OR_1, for DM without nephropathy and control groups. OR_2, for DM with nephropathy and control groups. P_1, significance between DM without nephropathy and control groups. P_2, significance between DM with nephropathy and control groups. *Means statistically significant.

Genetic susceptibility has been suggested as an important cofactor for the development and progression of DN [20,21]; therefore, detection of these genetic variants could help in the identification of those patients at high risk for DN.

In the present study, there was significant association of genotype frequency of the IGF-1 SNPs rs6214 and rs10860860 across the different groups of the study participants with some issues for consideration. We found that the highest frequency of variant allele GG in polymorphism rs6214 was present in patients with DN than the other groups (OR=20.57; 95% confidence interval, 2.25–74; *P*=0.001). Moreover, the frequency of variant AA in polymorphism rs10860860 was also found to be significantly higher among group with DN

Table 3 Frequency distribution of genotypes of insulin-like growth factor-1 single nucleotide polymorphism rs10860860 among the study groups

rs10860860	Control [*n* (%)]	DM without Nephropathy [*n* (%)]	DM with Nephropathy [*n* (%)]	OR_1	OR_2	P_1	P_2
AA	7 (28)	10 (38.5)	19 (73.1)*	0.29 (0.01–3.6)	7.4 (1.9–30.1)	0.25	0.001*
AT	15 (60)	14 (53.8)	6 (23.1)*	0.78 (0.2–2.4)	0.20 (0.05–0.8)	0.201	0.007*
TT	3 (12)	2 (7.7)	1 (38)	0.61(0.06–5.2)	0.3 (0.01–3.59)	0.48	0.28

Test used, χ^2. DM, diabetes mellitus; OR, odds ratio. OR_1, for DM without nephropathy and control groups. OR_2, for DM with nephropathy and control groups. P_2, significance between DM with nephropathy and control groups. P_1, significance between DM without nephropathy and control groups. *Means statistically significant.

Table 4 Association of genotypes of insulin-like growth factor-1 single nucleotide polymorphism rs6214 with laboratory parameters among all diabetics

rs6214	Cholesterol	Triglyceride	HDL	LDL	HbA1c	FBS	Creatinine	Uric acid	ACR
AA	253.31±38.9[a]	183.92±20.75	50.8±14.3	165.8±44.3	9.05±1.339	250.57±57.60	1.669±0.943[a]	6.8±1.2	518.33 ±864.83
GA	227.73±33.5	183.18±25.44	53±12.3	137.64±39.9[a]	9.17±1.639	251.59±60.68	1.672±0.963	6.8±2.1	300.68 ±508.6
GG	265.86±37.5	199.86±35.58	46±12.1	179.6±43.5[c]	8.6±1.433	257.56±32.02	3.278±2.724[b,c]	6.9±1.9	1280.39 ±1805.94
P	0.08	0.168	0.272	0.013	0.575	0.921	0.011	0.984	0.056

Data are presented as mean±SD. Test used, one way analysis of variance followed by post-hoc Tukey. ACR, albumin–creatinine ratio; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; FBS, fasting blood sugar; LDL, low-density lipoprotein. [a]Srs6214 Cholesterol Triglyceride HDL LDL HBA1c FBs Crea Uric acid ACR. [b]Significance between AA and GG. [c]Significance between GA and GG.

Table 5 Association of genotypes of insulin-like growth factor-1 single nucleotide polymorphism rs1080860 with laboratory parameters among all diabetics

rs1080860	TC	TG	HDL	LDL	HbA1c	FBS	Creatinine	Uric acid	ACR
AA	259.8±35[a]	195.3±32.1	48.3±12.6	172.2±39.8[a]	8.7±1.4	238.3±51.6	2.197±1.5	6.2±2.02[a]	1040.7±1465.4
AT	225.8±35.81	178.9±19.1[a]	53.2±13.1	136.72±43.8	8.9±1.3	274.5±36.6	1.8±1.2	4.9±1.3	166.4±382.8
TT	239±55.24	179.3±21.5	52±16.52	151.1±66.7	11.4±0.2[b,c]	254.7±101.8	4.4±5.7	4.9±0.4	138.2±160.1
P	0.010	0.122	0.430	0.025	0.010	0.052	0.059	0.037	0.053

Data are presented as mean±SD. Test used, one way analysis of variance followed by post-hoc Tukey. ACR, albumin–creatinine ratio; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride. [a]Significance between AA and AT. [b]Significance between AA and TT. [c]Significance between AT and TT.

(OR=7.37; 95% confidence interval, 1.87–30.07; *P*=0.001). Our finding could be owing to the prominent genetic role of IGF-1 in development of DN as suggested by Ewens *et al.* [22].In contrast to such evidence that imply a genetic role for IGF-1 in diabetic complications, Bazzaz *et al.* [3] found no association between IGF-1 gene polymorphisms and the development of late microangiopathic diabetic complications including DN. These conflicting results cannot necessarily rule out or account for the involvement of IGF-1 in the development of diabetic late complications; it can only be suggested that a pair of IGF-1 polymorphisms do not influence the development of those pathologies in the studied participants.

Another view from a statistical point of view, when the frequency of a genotype is low (as the GA and AT alleles polymorphism in the study group of DN), it generally weakens the power of that polymorphism as a cofactor, as such low frequency can be owing to its implied nonfunctionality and thus cannot be accounted as a phenotype modifier or an efficient genetic biomarker [23]. Another view that these alleles were associated with ORs less than 1 when compared with other groups, means that it could be significantly protective against DN. However, alleles that showed ORs more than 1 among diabetics with nephropathy could carry the risk predisposition for against DN [24].

We investigated the association between different alleles and laboratory parameters related to DN and found a genotype-dependent variation of the TC, LDL, uric acid, and serum creatinine, where these were found to be significantly higher in diabetics with genotypes GG and AA than other genotypes. However, the mean value of urinary ACR did not show significant differences between the genotypes. Parallel

to this result, Rietveld *et al.* [25] did not find any relationship between IGF-1 gene promoter polymorphism and renal function parameters. In our study, the observed relationship between IGF-1 genotype and metabolic laboratory parameters convenient with poorer renal function probably points to the increased risk of variants carriers to hazards of different metabolic effects.

The limitations in our study were small sample size, low-frequency polymorphic alleles, even in the case of their relevance and connection with the disease, so a large number of participants to identify their effect on the disease is required. Moreover, the study was not designed to investigate the differences in IGF-1 levels in relation to IGF-1 gene variants.

Conclusion

We observed that GG and AA alleles of IGF-1 gene polymorphism of patients with type 2 diabetes may modulate the susceptibility and/or progression of DN; moreover, we found a genotype-dependent variation of some metabolic parameters. So we concluded that the identification of these genetic variants at a biomarker level could allow the detection of those individuals at high risk for DN, which could thus help in the diagnosis, early prevention, and treatment of the disease. Further researches are requested to replicate our findings, owing to the probability of ethnic variation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Sesti G, Mannino GC, Andreozzi F, Greco A, Perticone M, Sciacqua A, *et al.* A polymorphism at IGF1 locus is associated with carotid intima media thickness and endothelium-dependent vasodilatation. Atherosclerosis 2014; 232:25–30.

2 Delafontaine P, Song YH, Li Y. Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. Arterioscler Thromb Vasc Biol 2004; 24:435–444.

3 Bazzaz J, Amoli MM, Taheri Z, Larijani B, Pravica V, Hutchinson IV. TGF-β1 and IGF-I gene variations in type 1 diabetes microangiopathic complications. J Diabetes Metab Disord 2014; 13:45.

4 Poulaki V, Joussen AM, Mitsiades N, Mitsiades CS, Iliaki EF, Adamis AP. Insulin-like growth factor-I plays a pathogenetic role in diabetic retinopathy. Am J Pathol 2004; 165:457–469.

5 Rietveld I. An insulin-like growth factor-I gene polymorphism modifies the risk of microalbuminuria in subjects with an abnormal glucose tolerance. Eur J Endocrinol 2006; 154:715–721.

6 Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol 2001; 54:311–316.

7 Varewijck AJ, Janssen JA. Insulin and its analogues and their affinities for the IGF1 receptor. Endocr Relat Cancer 2012; 19:F63–F75.

8 Lu L, Wang F, He L, Xue Y, Wang Y, Zhang H, *et al.* Interaction between IGF1 polymorphisms and the risk of acute lymphoblastic leukemia in Chinese children. Cell Physiol Biochem 2015; 36:1346–1358.

9 Kowalski A, Krikorian A, Lerma EV. Diabetic nephropathy for the primary care provider: new understandings on early detection and treatment. Ochsner J 2014; 14:369–379.

10 A strategy for arterial risk assessment and management in type 2 (non-insulin-dependent) diabetes mellitus. European Arterial Risk Policy Group on behalf of the International Diabetes Federation European Region. Diabet Med 1997; 14:611–621.

11 Ahmadi SA, Boroumand MA, Gohari-Moghaddam K, Tajik P, Dibaj SM. The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med 2006; 11:318–321.

12 Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20:470–475.

13 Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28:2077–2080.

14 Williams P, Robinson D, Bailey A. High-density lipoprotein and coronary risk factors in normal men. Lancet 1979; 313:72–75.

15 Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol 1969; 22:246.

16 Trivedi RC, Rebar L, Berta E, Stong L. New enzymatic method for serum uric acid at 500 nm. Clin Chem 1978; 24:1908–1911.

17 Vasiliades J. Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. Clin Chem 1976. 22:1664–1671.

18 Trivelli LA, Ranney HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971; 284:353–357.

19 Masao O, Fukushima H, Kulski JK, Inoko H. Single nucleotide polymorphism detection by polymerase chain restriction fragment length polymorphism. Nat Protocols 2:2857–2864.

20 Conserva F, Gesualdo L, Papale M. A Systems biology overview on human diabetic nephropathy: from genetic susceptibility to post-transcriptional and post-translational modifications. J Diabetes Res 2016.

21 Dorval G, Gribouval O, Martinez-Barquero V, Machuca E, Tete MJ, Baudouin V, *et al.* Clinical and genetic heterogeneity in familial steroid-sensitive nephrotic syndrome. Pediatr Nephrol 2017.

22 Ewens KG, George RA, Sharma K, Ziyadeh FN, Spielman RS. Assessment of 115 candidate genes for diabetic nephropathy by transmission/disequilibrium test. Diabetes 2005; 54:3305–3318.

23 Stephens RH, McElduff P, Heald AH, New JP, Worthington J, Ollier WE, Martin Gibson J. Polymorphisms in IGF-binding protein 1 are associated with impaired renal function in type 2 diabetes. Diabetes 2005; 54:3547–3553.

24 Freedman BI, Bostrom M, Daeihagh P, Bowden DW. Genetic factors in diabetic nephropathy. Clin J Am Soc Nephrol 2007; 2:1306–1316.

25 Rietveld I, Hofman A, Pols HAP, van Duijn CM, Lamberts SWJ, Janssen JAMJL. An insulin-like growth factor-I gene polymorphism modifies the risk of microalbuminuria in subjects with an abnormal glucose tolerance. Eur J Endocrinol 2006; 154:715–721.