

## Original Article

# Interaction between peroxisome proliferator- activated receptor gamma polymorphism and overweight on diabetic retinopathy in a Chinese case-control study

Yan Wang, Xin-Hua Wang, Ruo-Xi Li

Department of Ophthalmology, The Fourth people's Hospital of Shenyang City, Shenyang 110031, Liaoning Province, PR China

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**Abstract:** Background: Peroxisome proliferator-activated receptors  $\gamma$  (PPAR  $\gamma$ ) and overweight were both associated with diabetic retinopathy (DR), so the aim of this study was to investigate the association of four single nucleotide polymorphisms (SNPs) of PPAR  $\gamma$  with DR and additional role of gene-BMI interaction. Methods: A total of 500 patients with T2DM (236 men, 264 women), with a mean age of  $54.3 \pm 15.8$  years old, were selected, including 247 diabetic retinopathy patients and 253 controls. Four SNPs were selected for genotyping in the case-control study: rs1805192, rs709158, rs3856806, rs4684847. Logistic regression model was used to examine the interaction between SNP and overweight on DR, odds ratio (OR) and 95% confident interval (95% CI) were calculated. Results: The carriers of C allele of the rs1805192 polymorphism revealed decreased DR risk than those with Pro/Pro variants (Pro/Ala+Ala/Ala versus Pro/Pro, adjusted OR (95% CI)=0.86 (0.65-0.96),  $P=0.012$ ), after adjusting for covariates. We also found that obese subjects with Pro/Ala or Ala/Ala variants genotype have lowest DR risk, compared to obese subjects with Pro/Pro genotype or non- obese subjects with Pro/Ala or Ala/Ala (OR=0.40, 95% CI=0.32-0.63), after covariates adjustment. Conclusions: Our results support an important association between rs1805192 minor allele (Ala allele) of PPAR  $\gamma$  and DR, the interaction analysis shown a combined effect of Ala- BMI interaction on DR.

**Keywords:** Diabetic retinopathy, PPAR, polymorphism, overweight, interaction

## Introduction

Diabetic retinopathy (DR) is a long term complication of diabetes mellitus (DM) and a significant cause of blindness and ocular morbidity in developed nations. DR is characterized as a micro-vascular complication of diabetes and is always accompanied with other macro- and micro-vascular complications of diabetes [1-3]. DR is associated with both environmental and genetic factors. Several metabolic abnormalities are implicated in its pathogenesis. Some single nucleotide polymorphisms (SNPs) have been suggested as the candidate genes in DR etiology, such as peroxisome proliferator-activated receptor  $\gamma$  (PPAR  $\gamma$ ) family [4, 5], which is a nuclear transcription factor involved in adipocyte differentiation, glucose and lipid metabolism, and fatty acid transport. Some studies indicated that rs1805192 SNP plays a key role in regulating the expression of numerous genes involved in lipid metabolism, metabolic sy-

ndrome, inflammation, and atherosclerosis [6, 7]. Some environmental risk factors for DR are suggested, such as duration of diabetes, hypertension and so on. However, the association between body mass index (BMI) and DR is inconsistent [8-11]. Recently, lower DR prevalence was reported in patients with higher BMI [12, 13] in different populations, including Chinese. So the aim of this study was to investigate the association of PPAR  $\gamma$ , BMI and additional interaction on DR, based on a Chinese case-control study.

## Materials and methods

### Subjects

This was a case-control study. Chinese patients with type 2 diabetes (T2DM) were consecutively recruited between January 2013 and December 2014 from the Fourth people's hospital of Shenyang City in Liaoning Province, China. A

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**Table 1.** Probe sequence for four SNPs used for Taqman fluorescence probe analysis

SNP	rs number	Chromosome	Position	Probe sequence
C1341T	rs3856806	3	12415557	5'-GGTTGACACAGAGATGCCATTCTGG[C/G]CCACCAACTTTGGGATCAGCTCCGT-3'
Intron A>G	rs709158	3	12403176	5'-AGATACGGGGGAGGAAATCACTGG[A/G]TTTTACAATATATTTTTCAAGGCAA-3'
Pro12Ala	rs1805192	3	12361238	5'-ACCTCAGACAGATTGTCACGGAACA[C/T]GTGCAGCTACTGCAGGTGATCAAGA-3'
Intron C>T	rs4684847	3	12326337	5'-ATTTATTTAAATCATCTCTAATTC[C/T]ACAACCTCCGAAAGATAAGAAAACA-3'

**Table 2.** General characteristics of study participants in case and control group

Variables	Total (n=500)	DR cases group (n=247)	Control group (n=253)	P-values
Age (years)	54.3 ± 15.8	53.70 ± 16.8	55.4 ± 16.2	0.320
Males N (%)	236 (47.2)	112 (45.3)	124 (49.0)	0.411
Smoke N (%)	176 (35.2)	92 (37.2)	84 (33.2)	0.399
Alcohol consumption N (%)	280 (56.0)	144 (58.3)	136 (53.8)	0.356
Duration of diabetes	7.3 ± 3.7	8.6 ± 4.8	6.4 ± 4.3	<0.001
High fat diet N (%)	210 (42.0)	106 (42.9)	104 (41.1)	0.751
Low fiber diet N (%)	204 (40.8)	108 (43.7)	106 (41.9)	0.752
High salt diet N (%)	136 (27.2)	71 (28.7)	65 (25.7)	0.514
Occupational activity N (%)				0.273
Full mental labour	63 (12.6)	34 (13.8)	29 (11.5)	
Main mental labour	108 (21.6)	50 (20.2)	58 (22.9)	
Main physical labour	203 (40.6)	93 (37.7)	110 (43.5)	
Full physical labour	126 (25.2)	70 (28.3)	56 (22.1)	
WC (cm)	86.9 ± 6.4	86.1 ± 10.8	87.8 ± 10.5	0.075
BMI (kg/m <sup>2</sup> )	26.1 ± 6.5	25.8 ± 6.7	27.3 ± 6.2	0.009
FPG (mmol/L)	8.3 ± 1.9	8.4 ± 2.6	8.2 ± 2.3	0.362
TG (mmol/L)	1.9 (1.2-2.4)	2.1 (1.2-2.3)	1.8 (1.1-2.5)	0.278
TC (mmol/L)	4.90 ± 1.12	5.4 ± 1.1	4.8 ± 1.1	<0.001
HDL (mmol/L)	1.29 ± 0.30	1.24 ± 0.33	1.31 ± 0.27	0.009

Note: median and inter quartile for TG; means ± standard deviation for age, FPG, TC, HDL-C; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; FPG, fast plasma glucose; TG, triglyceride.

total of 500 patients with T2DM (236 men, 264 women), with a mean age of 54.3 ± 15.8 years old, were selected, including 247 diabetic retinopathy patients and 253 controls. Informed consent was obtained from all patients and healthy donors.

### Body measurements

Data on demographic information, lifestyle risk factors for all participants were obtained using a standard questionnaire administered by trained staffs. Body weight, height, was measured, and BMI was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were collected in the morning after at least 8 hours of fasting. All plasma and serum samples were frozen at -80°C until laboratory testing. Plasma glucose was measured using an oxidase enzymatic method. All analysis was performed by the

same lab. The method of investigation at the follow up was same as that at baseline.

### Genomic DNA extraction and genotyping

Four SNPs were selected for genotyping in the case-control study: rs1805192, rs709158, rs3856806, rs4684847. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Four SNPs were detected by Taqman fluorescence probe. Probe sequences of four SNPs were shown in **Table 1**. ABI Prism7000 software and allelic discrimination procedure was used for genotyping of fore-mentioned four SNPs. A 25 µl reaction mixture including 1.25 µl SNP Genotyping Assays (20×), 12.5 µl Genotyping Master Mix (2×), 20ng DNA, and the conditions were as follows: initial denaturation for 10 min and 95°C, denaturation for

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**Table 3.** Genotype and allele frequencies of four SNPs between case and control group

SNPs	Genotypes and Alleles	Frequencies N (%)		OR (95% CI) <sup>a</sup>	P-values	
		Case (n=247)	Control (n=253)			
rs3856806	CC	125 (50.6)	131 (51.8)	1.00	0.858	
	CT	100 (40.5)	97 (38.3)	0.92 (0.66-1.22)		
	TT	22 (8.9)	25 (9.8)	1.03 (0.64-1.71)		
	CT+TT	122 (49.4)	122 (48.1)	0.95 (0.73-1.26)	0.840	
	C	350 (70.8)	359 (70.9)			0.941
rs709158	T	144 (29.2)	147 (29.1)		0.356	
	AA	120 (48.6)	126 (49.8)	1.00		
	AG	93 (37.6)	90 (35.6)	1.12 (0.97-1.47)		
	GG	34 (13.8)	37 (14.6)	0.98 (0.83-1.31)		
	AG+GG	127 (51.4)	117 (50.2)	1.06 (0.92-1.43)		0.433
rs1805192	A	333 (67.4)	342 (67.6)		0.156	
	G	161 (32.6)	164 (32.4)			
	PP	144 (58.3)	118 (46.6)	1.00		0.010
	PA	85 (34.4)	100 (39.5)	0.92 (0.71-1.24)		
	AA	18 (7.3)	35 (13.8)	0.81 (0.62-0.93)		
PA+AA	103 (41.7)	135 (53.3)	0.86 (0.65-0.96)	0.012		
rs4684847	Pro	373 (75.5)	336 (66.4)		0.002	
	Ala	121 (24.5)	170 (33.6)			
	CC	154 (62.3)	160 (63.2)	1.00		0.92
	CT	73 (29.6)	78 (30.8)	0.98 (0.71-1.36)		
	TT	20 (8.1)	15 (5.9)	1.07 (0.80-1.36)		
CT+TT	189 (36.9)	93 (36.7)	1.02 (0.78-1.32)	0.96		
	C	381 (77.1)	398 (78.6)		0.34	
	T	113 (22.9)	108 (21.4)			

<sup>a</sup>Adjusted for gender, age, smoke and alcohol status, high fat diet, low fiber diet, TC, HDL.

15 s and 92°C, annealing and extension for 90 s and 60°C, 50 cycles.

### Diagnostic criteria

Diagnosis of diabetes at baseline for a fasting glucose was  $\geq 126$  mg/dl (7.0 mmol/l) or if hypoglycemic therapy (oral agents or insulin) had been executed. During the follow-up, the criteria for the diagnosis of T2DM included a fasting glucose  $\geq 126$  mg/dl (7.0 mmol/l), or a 2 h postprandial blood glucose  $\geq 200$  mg/dl (11.0 mmol/l), or if hypoglycemic therapy (oral agents or insulin) had been started in the interim.

DR [14] was diagnosed with the presence of retinal hemorrhages, exudates and macular edema. Neuropathy was diagnosed in the presence of persistent numbness, paresthesia, loss of hearing of the tuning fork and sense of vibration.

Overweight was defined by using WHO criteria for Asian populations: BMI value  $\geq 24$  kg/m<sup>2</sup> [15].

### Statistical analysis

The mean and SD for normally distributed continuous variables, and percentages for categorical variable, were calculated and compared between case and control group participants. The genotype and allele frequencies were obtained by direct count. Genotype distributions in DR patients and controls were evaluated by  $\chi^2$  test using SPSS (version 19.0; SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium (HWE) was performed by using SNPStats (available online at <http://bioinfo.iconcologia.net/SNPstats>). Logistic regression model was used to examine the interaction between SNP and overweight on DR, odds ratio (OR) and 95% confident interval (95% CI) were calculated. Odds were adjusted for gender, age, smoke and alcohol status, high fat diet, low fiber diet, TC, high density lipoprotein (HDL).

### Results

A total of 500 patients with T2DM (236 men, 264 women), with a mean age of  $54.3 \pm 15.8$  years old, were selected, including 247 diabetic retinopathy patients and 253 controls. Participants characteristics stratified by cases and controls are shown in **Table 2**. The distributions of males, smoking, alcohol consumption, high fat diet, low fiber diet, high salt diet and occupational activity were not different between cases and controls. The mean of duration of diabetes, BMI, TC and HDL were significantly different between cases and controls.

All genotypes were distributed according to Hardy-Weinberg equilibrium. There were significant differences in rs1805192 alleles and genotypes distributions between cases and controls (**Table 3**). The frequencies for Ala allele of

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**Table 4.** Interaction analysis for rs1805192 and overweight on DR by using logistic regression

rs1805192	Overweight	Number of subjects	OR (95% CI) <sup>a</sup>	P-values
PP	No	104	1.00	-
PA or AA	No	72	0.91 (0.68-1.10)	0.105
PP	Yes	30	0.82 (0.59-0.93)	0.010
PA or AA	Yes	31	0.40 (0.32-0.63)	<0.001

<sup>a</sup>Adjusted for gender, age, smoke and alcohol status, high fat diet, low fiber diet, TC and HDL-C. P: Pro; A: Ala.

rs1805192 was higher in controls (33.6% in controls and 24.5% in DR subjects,  $P=0.002$ ). Logistic analysis showed that the association between genotypes of variants in rs1805192 and decreased DR risk, after adjustment for gender, age, smoke and alcohol status, high fat diet, low fiber diet, TC, HDL, DR risk was significantly lower in individuals with rs1805192-Ala allele ( $P$  values<0.05). The carriers of C allele of the rs1805192 polymorphism revealed decreased DR risk than those with Pro/Pro variants (Pro/Ala+Ala/Ala versus Pro/Pro, adjusted OR (95% CI)=0.86 (0.65-0.96),  $P=0.012$ ). However, we did not find any significant association between other three SNPs and DR before or after covariates adjustment.

In order to obtain the odds ratios and 95% CI for the joint effects of rs1805192 genotype and overweight on DR, we conducted interaction analysis between rs1805192 and overweight. We found that obese subjects with Pro/Ala or Ala/Ala genotype have lowest DR risk, compared to obese subjects with ProPro genotype or non-obese subjects with Pro/Ala or Ala/Ala (OR=0.40, 95% CI=0.32-0.63), after adjustment for gender, age, smoke and alcohol status, high fat diet, low fiber diet, TC and HDL-C (Table 4).

### Discussion

The result of this study indicated that rs1805192 minor allele (Ala allele) of PPAR  $\gamma$  is significantly associated with lower DR risk. However, we did not find any association between the others SNPs (rs709158, rs3856806, rs4684847) and DR. Although many studies have taken a candidate gene approach to investigate the genetic etiology of DR, implicating the potential candidate genes in DR etiology, which include genes for rs1805192 polymorphism of PPAR  $\gamma$  [16], however the results of

association between rs1805192 and DR are inconsistent. Zhang et al. [17] indicated that no significant associations were found between polymorphisms in the PPAR  $\gamma$  genes and DR or PDR in a Chinese T2DM population. However, Tariq et al. [18] reported a protective role of the 12Ala polymorphism against proliferative DR in individuals with T2DM in Pakistan. Malecki et al. [16] indicated that the Ala variant of the rs1805192 polymorphism of PPAR gamma might be associated with decreased risk of DR in T2DM. This effect may be indirect, at least in part, due to diabetic kidney disease. In our study, we obtained the same results with these studies.

DR is associated with both environmental and genetic factors. Several metabolic abnormalities are implicated in its pathogenesis, such as duration of diabetes, glycaemic control, hypertension, and other environmental factors. Recently, more and more studies focused on the association between obesity/overweight and DR. Although Sen et al. [19] did not obtained a significant association between BMI and DR. Lu et al. [20] indicated that overweight patients have lower DR prevalence than normal weight individuals, which may be attributable to better  $\beta$  cell function in overweight patients. Abougalambou et al. [21] also suggested that BMI was negatively associated with diabetic retinopathy among type 2 diabetes patients at teaching hospital in Malaysia. In this study, we found that the DR cases have lower BMI level. In the interaction analysis, we also found a significant interaction between rs1805192 and overweight on DR.

Tawfik et al. [22] indicated that Suppression of PPAR  $\gamma$  is involved in the pathogenesis of diabetic retinopathy, suppression of PPAR  $\gamma$  is downstream from NADPH oxidase activation in diabetic and ischemic retinopathies. Suppression of PPAR  $\gamma$  leads to activation of the NF- $\kappa$ B signaling pathway, including the up regulation of intercellular adhesion molecule-1 (ICAM-1), increased leukocyte-endothelial interaction, and retinal vascular dysfunction, which could be beneficial in preventing retinal vascular damage induced by hyperglycemia. Increased BMI was associated with greater pancreatic  $\beta$  cell mass and higher C-peptide (CP) levels [22, 23]. Moreover, higher fasting C-peptide (FCP)

and postprandial CP reduce DR risk in both type 1 and type 2 diabetes [24-26]. Therefore, it is plausible that the coexistence of Ala allele of PPAR  $\gamma$  and overweight contribute to the lowest DR risk, and as observed in this study.

Several limitations of this study should be considered. Firstly, only four SNPs of PPAR  $\gamma$  gene were chosen. The limited SNPs were not sufficient to capture most genetic information of PPAR  $\gamma$ , more SNPs should be included in the further studies. Secondly, there was a relatively small sample size in the study, though the number of study participants met the requirement for analysis, other larger sample studies should be conducted in the future. Thirdly, we did not obtain any information on sugar dietary, which should be included in the analysis of future study.

In conclusion, our results support an important association between rs1805192 minor allele (Ala allele) of PPAR  $\gamma$  and DR, the interaction analysis shown a combined effect of Ala- BMI interaction between rs1805192 and overweight on DR.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Ruo-Xi Li, The Fourth people's Hospital of Shenyang City, Huang He Nan Street, Huanggu District, Shenyang 110031, Liaoning Province, PR China. Tel: +86-024-2622-7130; E-mail: liruoxi3313@163.com

### References

- [1] Karlberg C, Falk C, Green A, Sjolie AK, Grauslund J. Proliferative retinopathy predicts nephropathy: a 25-year follow-up study of type 1 diabetic patients. *Acta Diabetol* 2012; 49: 263-268.
- [2] Pradeepa R, Surendar J, Indulekha K, Chella S, Anjana RM, Mohan V. Relationship of diabetic retinopathy with coronary artery disease in Asian Indians with type 2 diabetes: The Chennai Urban Rural Epidemiology Study (CURES) Eye Study-3. *Diabetes Technol Ther* 2014; 17: 112-8.
- [3] Miyamoto M, Kotani K, Okada K, Fujii Y, Konno K, Ishibashi S, Taniguchi N. The correlation of common carotid arterial diameter with atherosclerosis and diabetic retinopathy in patients with type 2 diabetes mellitus. *Acta Diabetol* 2012; 49: 63-68.
- [4] Ma J, Li Y, Zhou F, Xu X, Guo G, Qu Y. Meta-analysis of association between the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor- $\gamma$ 2 gene and diabetic retinopathy in Caucasians and Asians. *Molecular Vision* 2012; 18: 2352-2360.
- [5] Treacy MP, Hurst TP. The case for intraocular delivery of PPAR agonists in the treatment of diabetic retinopathy. *BMC Ophthalmol* 2012; 12: 46.
- [6] Argmann CA, Cock TA, Auwerx J. Peroxisome proliferator-activated receptor gamma: the more the merrier? *Eur J Clin Invest* 2005; 35: 82-92.
- [7] Desvergne B. Be fit or be sick: peroxisome proliferator-activated receptors are down the road. *Mol Endocrinol* 2004; 18: 1321-32.
- [8] Henricsson M, Nystrom L, Blohme G, Ostman J, Kullberg C, Svensson M, Schölin A, Arnqvist HJ, Björk E, Bolinder J, Eriksson JW, Sundkvist G. The incidence of retinopathy 10 years after diagnosis in young adult people with diabetes: results from the nationwide population-based diabetes incidence study in Sweden (DISS). *Diabetes Care* 2003; 26: 349-54.
- [9] Zhang L, Krzentowski G, Albert A, Lefebvre PJ. Risk of developing retinopathy in diabetes control and complications trial type 1 diabetic patients with good or poor metabolic control. *Diabetes Care* 2001; 24: 1275-1279.
- [10] Dirani M, Xie J, Fenwick E, Benarous R, Rees G, Wong TY, Lamoureux EL. Are obesity and anthropometry risk factors for diabetic retinopathy? The diabetes management project. *Invest Ophthalmol Vis Sci* 2011; 52: 4416-4421.
- [11] van Leiden HA, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Polak BC. Risk factors for incident retinopathy in a diabetic and nondiabetic population: the Hoorn study. *Arch Ophthalmol* 2003; 121: 245-251.
- [12] Rooney D, Lye WK, Tan G, Lamoureux EL, Ikram MK, Cheng CY, Kumari N, Zheng YF, Mitchell P, Wang JJ, Wong TY, Sabanayagam C. Body mass index and retinopathy in Asian populations with diabetes mellitus. *Acta Diabetol* 2014; 52: 73-80.
- [13] Raman R, Rani PK, Gnanamoorthy P, Sudhir RR, Kumaramanikavel G, Sharma T. Association of obesity with diabetic retinopathy: Sankara Nethralaya diabetic retinopathy epidemiology and molecular genetics study (SN-DREAMS Report no. 8). *Acta Diabetol* 2010; 47: 209-215.



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- [14] Watkins PJ. Retinopathy. *BMJ* 2003; 326: 924-926.
- [15] Report of a WHO Consultation. Obesity: Preventing and managing the global epidemic. WHO Technical Report Series, Geneva 2000; 894: i-xii, 1-253.
- [16] Malecki MT, Cyganek K, Mirkiewicz-Sieradzka B, Wolkow PP, Wanic K, Skupien J, Solnica B, Sieradzki J. Alanine variant of the Pro12Ala polymorphism of the PPAR gamma gene might be associated with decreased risk of diabetic retinopathy in type 2 diabetes. *Diabetes Res Clin Pract* 2008; 80: 139-45.
- [17] Zhang Y, Meng N, Lv Z, Li H, Qu Y. The gene polymorphisms of UCP1 but not PPAR  $\gamma$  and TCF7L2 are associated with diabetic retinopathy in Chinese type 2 diabetes mellitus cases. *Acta Ophthalmol* 2015; 93: e223-9.
- [18] Tariq K, Malik SB, Ali SH, Maqsood SE, Azam A, Muslim I, Khan MS, Azam M, Waheed NK, Qamar R. Association of Pro12Ala polymorphism in peroxisome proliferator activated receptor gamma with proliferative diabetic retinopathy. *Molecular Vision* 2013; 19: 710-717.
- [19] Sen D, Ghosh S, Roy D. Correlation of C-reactive protein and body mass index with diabetic retinopathy in Indian population. *Diabetes Metab Syndr* 2015; 9: 28-9.
- [20] Lu J, Hou X, Zhang L, Jiang F, Hu C, Bao Y, Jia W. Association between body mass index and diabetic retinopathy in Chinese patients with type 2 diabetes. *Acta Diabetol* 2015; 52: 701-8.
- [21] Abougambou SS, Abougambou AS. Risk factors associated with diabetic retinopathy among type 2 diabetes patients at teaching hospital in Malaysia. *Diabetes Metab Syndr* 2015; 9: 98-103.
- [22] Tawfik A, Sanders T, Kahook K, Akeel S, Elmarakby A, Al-Shabrawey M. Suppression of Retinal Peroxisome Proliferator-Activated Receptor G in Experimental Diabetes and Oxygen-Induced Retinopathy: Role of NADPH Oxidase. *Invest Ophthalmol Vis Sci* 2009; 50: 878-84.
- [23] Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab* 2008; 10 Suppl 4: 32-42.
- [24] Lachin JM, McGee P, Palmer JP. Impact of C-peptide preservation on metabolic and clinical outcomes in the diabetes control and complications trial. *Diabetes* 2014; 63: 739-748.
- [25] Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 2003; 26: 832-836.
- [26] Panero F, Novelli G, Zucco C, Fornengo P, Perotto M, Segre O, Grassi G, Cavallo-Perin P, Bruno G. Fasting plasma C-peptide and micro- and macrovascular complications in a large clinic-based cohort of type 1 diabetic patients. *Diabetes Care* 2009; 32: 301-305.