Gene–gene interaction of erythropoietin gene polymorphisms and diabetic retinopathy in Chinese Han

YanFei Fan, Yin-Yu Fu, Zhi Chen, Yuan-Yuan Hu and Jie Shen

Department of Endocrinology and Metabolism, the Third Affiliated Hospital of Southern Medical University, No. 183, West Zhongshan Rd, Guangzhou 510500, China

Corresponding author: Jie Shen. Email: Drshenjie33@163.com

Abstract

The aim of this study was to investigate the association of three single nucleotide polymorphisms in the erythropoietin gene polymorphisms with diabetic retinopathy and additional role of gene–gene interaction on diabetic retinopathy risk. A total of 1193 patients (579 men, 614 women) with type 2 diabetes mellitus were selected, including 397 diabetic retinopathy patients and 796 controls (type 2 diabetes mellitus patients without diabetic retinopathy); the mean age of all participants was 56.7 ± 13.9 years. Three single nucleotide polymorphisms were selected: rs507392, rs1617640, and rs551238. The *t*-test was used for comparison of erythropoietin protein level erythropoietin levels in patients having different erythropoietin genotypes. Logistic regression model was used to examine the association between three single nucleotide polymorphisms and diabetic retinopathy. Odds ratio (OR) and 95% confident interval (95% CI) were calculated. Generalized multifactor dimensionality reduction was employed to analyze the impact of interaction among three single nucleotide polymorphisms on CVD risk. After covariates adjustment, the carriers of homozygous mutant of three single nucleotide polymorphisms have higher diabetic retinopathy risk than those with wild-type homozygotes, OR (95% CI) were 2.04 (1.12–2.35), 1.87 (1.10–2.41) and 1.15 (1.06–1.76), respectively. Generalized multifactor dimensionality reduction model indicated a significant three-locus model ($p = 0.0010$) involving rs507392, rs1617640, and rs551238. Overall, the three-locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 60.72%. Subjects with TC or CC-TG or GG-AC or CC genotype have the highest diabetic retinopathy risk. In conclusion, our results support an important association of rs507392, rs1617640 and rs551238 minor allele of erythropoietin with increased diabetic retinopathy risk, and additional interaction among three single nucleotide polymorphisms.

Keywords: Diabetic retinopathy, EPO, polymorphism, interaction

Experimental Biology and Medicine 2016; 241: 1524–1530. DOI: 10.1177/1535370216645210

Introduction

Diabetic retinopathy (DR), which is a long-term complication of diabetes mellitus (DM) and a main cause of blindness and ocular morbidity, is characterized as a micro-vascular complication of diabetes and is always accompanied with other micro-vascular complications of diabetes.^{1–3} The mechanisms underlying DR are still incompletely understood. Evidence suggested that damage for retinal blood vessels by long-standing hyperglycemia can lead to retinal hypoxia, which could stimulate the DNA-binding activity of hypoxia-inducible factor. Studies^{4,5} have suggested that the development of DR has been shown to have strong genetic components. In addition, vascular endothelial growth factor (VEGF), erythropoietin (EPO), interleukin-6 and advanced glycation end products are involved in this etio-pathogenesis of DR,^{6–9} and EPO is considered to have an angiogenic potential equivalent to VEGF.¹⁰

EPO was a glycoprotein which plays a major role in stimulation of bone marrow stem cells and erythropoiesis. The expression of EPO receptors in the retina¹¹ and vascular endothelial cells¹² has been demonstrated in previous studies. Some studies also reported that concentration of EPO in the vitreous of patients with DM and proliferative DR (PDR) risk was higher than that in controls.^{10,12,13} Recently, some studies have focused on the association between EPO gene and DR risk; however, the results of these studies were inconsistent.^{14–17} In addition, it was known that genetic susceptibility to DR was related to several genes; however, till now no studies involved in the impact of interaction among many single nucleotide polymorphisms (SNPs) of EPO gene on DR were reported. So the aim of this study was to investigate the impact of association of three SNPs in the EPO gene polymorphisms, and

Table 1 Description and probe sequence for 5 SNPs used for Taqman fluorescence probe analysis

rs number	Chromosome	Functional Consequence	Probe sequence
rs507392	7:100722313	Intron variant	5'-ATCTGGGACTACCACCATGCACCAC[C/T]TCACCTGACTAATTTTTTAAATGT-3'
rs1617640	7:100719675	Upstream variant 2kb	5'-TGGCTTCTGGAAACCTGAGCCAGA[G/T]GAGTGAGATCCCAGAGCAGGAGAC-3'
rs551238	7:100723905	Downstream variant 500b	5'-TACTGCGGTGAGGCCTGAATGGAG[A/C]CACCTTATTGACCAGCGTAGGCAGA-3'

additional role of multiple SNPs' interaction on DR risk, 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 February 2011 and October 2013 from the third affiliated hospital of southern medical university, China. The T2DM patients with DR were included in the case group, and T2DM patients without DR were included in the control group. The patients with end-stage renal disease (ESRD) were excluded. At last, a total of 1193 T2DM patients (579 men, 614 women), with a mean age of 56.7 ± 13.9 years, were selected, including 397 DR patients and 796 controls. All subjects were examined by ophthalmic examinations, including best-corrected visual acuity, slit-lamp bio microscopy, and fundus examination. All examinations were performed by the same ophthalmologist. Blood samples were collected from each participant. Written informed consent was obtained from each individual prior to participation in the study.

Body measurements

Data on demographic information, lifestyle risk factors and duration of diabetes for all participants were obtained using a standard questionnaire administered by trained staffs. Body weight, height, and waist circumference (WC) were measured according to standardized procedures.¹⁸ Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Two times of sitting blood pressure (BP) measurements were taken at 30-s intervals by trained observers using a standard mercury sphygmomanometer after the subjects had been resting for 5 min according to a standard protocol. The first and the fifth Korotkoff sounds were recorded as the SBP (systolic blood pressure) and DBP (diastolic blood pressure), respectively. The mean of the two BP measurements was used in the analysis. Blood samples were collected in the morning after at least 8 h of fasting.

All plasma and serum samples were frozen at -80°C until laboratory testing. Fasting plasma glucose (FPG) was measured using an oxidase enzymatic method. Concentrations of high-density lipoprotein (HDL)-cholesterol and triglyceride (TG) were assessed enzymatically by an automatic biochemistry analyzer (Hitachi Inc, Tokyo, Japan) using commercial reagents. All analysis was performed by the same lab.

Genomic DNA extraction and genotyping

We selected SNPs which have been reported that it was associated with DR, such as rs1617640 and rs551238 in EPO. We also selected some SNPs, which were not reported or few reported in previous studies, such as rs507392. Three SNPs were investigated in the current case-control study: rs507392, rs1617640, and rs551238. 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. Three SNPs were detected by Taqman fluorescence probe. Probe sequences of three SNPs were shown in Table 1. ABI Prism7000 software and allelic discrimination procedure were used for genotyping of aforementioned three SNPs. A 25 μl reaction mixture including 1.25 μl SNP Genotyping Assays (20 $\times$), 12.5 μl Genotyping Master Mix (2 $\times$), 20 ng DNA, and the conditions were as follows: initial denaturation for 10 min and 95°C , denaturation for 15 s and 92°C , annealing and extension for 90 s and 60°C , 50 cycles.

Diagnostic criteria

The criteria for the diagnosis of T2DM included a fasting glucose ≥ 126 mg/dl (7.0 mmol/l), or a 2 h postprandial blood glucose ≥ 200 mg/dl (11.0 mmol/l), or if hypoglycemic therapy (oral agents or insulin) had been started in the interim.

Diabetic retinopathy (DR)¹⁹ 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.

Statistical analysis

The mean and standard deviation (SD) were calculated for normally distributed continuous variables, and percentages were calculated for categorical variables. The genotype and allele frequencies were obtained by direct count. Genotype distributions in DR patients and controls were evaluated by χ^2 test using SPSS (version 19.0; SPSS Inc., Chicago, IL, USA). The *t*-test was used for comparison of EPO protein level EPO levels in patients having different EPO genotypes. 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 impact of interaction among three SNPs on DR. Odds ratio (OR) and 95% confident interval (95%CI) were calculated. Odds were adjusted for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC and HDL.

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

Variables	Total (n = 1193)	DR cases group (n = 397)	Control group (n = 796)	P values
Age (years)	56.7 ± 13.9	56.1 ± 14.2	56.9 ± 14.1	0.357
Males N (%)	579 (48.5)	187 (47.1)	392 (49.2)	0.534
Smoke N (%)	421 (35.3)	148 (37.3)	273 (34.2)	0.321
Alcohol consumption N (%)	544 (45.6)	199 (50.1)	345 (43.3)	0.046
Duration of DM (years)	6.8 ± 2.7	9.5 ± 3.8	5.7 ± 3.0	<0.001
High fat diet N (%)	496 (41.6)	174 (43.8)	322 (40.4)	0.407
Low fiber diet N (%)	487 (40.8)	173 (43.6)	314 (39.4)	0.184
WC (cm)	83.9 ± 8.4	82.9 ± 9.8	84.1 ± 9.3	0.039
BMI (kg/m ²)	24.8 ± 6.5	23.4 ± 6.7	25.3 ± 6.2	<0.001
FPG (mmol/L)	8.3 ± 1.9	8.4 ± 2.6	8.2 ± 2.3	0.176
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 ± 1.1	5.0 ± 1.3	4.9 ± 1.2	0.187
HDL (mmol/L)	1.27 ± 0.30	1.24 ± 0.33	1.28 ± 0.27	0.025
SBP (mmHg)	142.7 ± 16.8	150.3 ± 20.6	139.7 ± 18.2	<0.001
DBP (mmHg)	86.3 ± 14.2	97.4 ± 16.8	82.0 ± 15.1	<0.001
EPO protein level (IU/L)	18.8 ± 5.1	23.2 ± 6.3	14.2 ± 5.7	<0.001

Note: median and inter quartile for TG; means ± standard deviation for age, duration of DM, WC, BMI, FPG, TC, HDL-C, SBP, DBP, and EPO protein level; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; FPG, fast plasma glucose; TG, triglyceride; WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Generalized multifactor dimensionality reduction (GMDR)²⁰ was employed to investigate the interaction among three SNPs. Some parameters were calculated, including cross-validation consistency, the testing balanced accuracy, and the sign test, to assess each selected interaction. The cross-validation consistency score is a measure of the degree of consistency with which the selected interaction is identified as the best model among all possibilities considered. The testing balanced accuracy is a measure of the degree to which the interaction accurately predicts case-control status with scores between 0.50 (indicating that the model predicts no better than chance) and 1.00 (indicating perfect prediction). Finally, a sign test or a permutation test (providing empirical *P* values) for prediction accuracy can be used to measure the significance of an identified model.

Results

A total of 1193 patients with T2DM (579 men, 614 women) were selected, including 397 diabetic retinopathy patients and 796 controls, and the mean age of all participants was 56.7 ± 13.9 years. Participant characteristics stratified by cases and controls are shown in Table 2. The distribution of alcohol consumption was significantly different between cases and controls. The mean of duration of diabetes, BMI, WC, HDL, EPO level, SBP, and DBP was significantly different between cases and controls. We also compared EPO protein level in the different genotypes in three SNPs, and we found that the EPO protein level was the highest in subjects with mutation type homozygous genotype in the three SNPs (all *P* values were less than 0.001) (Figure 1).

There were significant differences in rs507392, rs1617640, and rs551238 alleles and genotypes distributions between cases and controls (Table 3). The frequencies for three SNP

minor alleles were higher in cases than that in controls, and C allele of rs507392 was 22.7% in controls and 28.8% in DR patients (*p* = 0.001), and G allele of rs1617640 was 22.2% in controls and 27.3% in DR patients (*p* = 0.006), and C allele of rs551238 was 29.2% in controls and 24.4% in DR patients (*p* = 0.014). Logistic analysis showed a significant association between genotypes of variants in three SNP and increased DR risk, after adjustment for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC and HDL. The carriers of homozygous mutant of three SNP polymorphism have higher DR risk than those with wild-type homozygotes, and OR (95% CI) was 2.04 (1.12–2.35), 1.87 (1.10–2.41), and 1.15 (1.06–1.76), respectively.

We employed the GMDR analysis to assess the impact of the interaction among three SNPs on DR risk, after adjustment for covariates including gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC, and HDL. Table 4 summarizes the results obtained from GMDR analysis for one- to three-locus models. There was a significant three-locus model (*p* = 0.0010) involving rs507392, rs1617640, and rs551238, indicating a potential gene-gene interaction among rs507392, rs1617640, and rs551238. Overall, the three-locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 60.72%.

In order to obtain the odds ratios and 95%CI for the interaction among rs507392, rs1617640, and rs551238, we conducted interaction analysis among three SNPs by using logistic regression. We found that subjects with TC or CC-TG or GG-AC or CC genotype have the highest DR risk, compared to subjects with TT-TT-AA genotype, OR (95% CI) was 3.84 (1.75–8.33), after adjustment for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC, and HDL (Table 5).

Figure 1 Comparison of EPO protein level in the different genotypes in three SNPs. (A color version of this figure is available in the online journal.)

Table 3 Genotype and allele frequencies of three SNP between case and control group

SNPs	Genotypes and alleles	Frequencies N (%)		OR (95% CI)*	P-values
		Case (n = 397)	Control (n = 796)		
rs507392	TT	202 (50.9)	463 (58.2)	1.00	<0.001
	TC	161 (40.6)	305 (38.3)	1.03 (0.76–1.22)	
	CC	34 (8.6)	28 (3.5)	2.04 (1.12–2.35)	
	CC + TC	195 (49.2)	333 (41.8)	1.58 (1.06–1.97)	0.018
	T	565 (71.2)	1231 (77.3)		0.001
	C	229 (28.8)	361 (22.7)		
rs1617640	TT	208 (52.4)	468 (58.8)	1.00	0.004
	TG	161 (40.6)	302 (37.9)	1.04 (0.78–1.24)	
	GG	28 (7.0)	26 (3.2)	1.87 (1.10–2.41)	
	TG + GG	189 (47.6)	328 (41.1)	1.46 (1.05–1.87)	0.038
	T	577 (72.7)	1238 (77.8)		0.006
	G	217 (27.3)	354 (22.2)		
rs551238	AA	203 (51.1)	452 (56.8)	1.00	0.022
	AC	156 (39.3)	299 (37.6)	1.02 (0.72–1.37)	
	CC	38 (9.6)	45 (5.6)	1.15 (1.06–1.76)	
	CC + AC	194 (48.9)	344 (43.2)	1.06 (0.98–1.44)	0.071
	A	562 (70.8)	1203 (75.6)		0.014
	C	232 (29.2)	389 (24.4)		

*Adjusted for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC and HDL.

Discussion

In the present study, we found that there was a significant association between EPO genotypes of variants in three SNPs and increased DR risk. There were higher DR risks in the carriers of C allele of 507392, G allele of rs1617640 and C allele of rs551238, suggesting that variants in three SNP were associated with increased DR risk. The human EPO gene is

located on chromosome 7q21. Previous studies have focused on the relation of EPO gene and DR; however, the results were inconsistent. Balasubbu et al.¹⁷ indicated that rs1617640 was not associated with DR, which was consistent with the results of study for Chinese population.¹⁶ In contrast to the aforementioned results, Abhary et al.¹⁴ indicated that

Table 4 Best gene–gene interaction models, as identified by GMDR

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	P values*
1	rs507392	9/10	0.5669	0.0107
2	rs507392 rs1617640	8/10	0.5399	0.0547
3	rs507392 rs1617640 rs551238	10/10	0.6072	0.0010

*Adjusted for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC, and HDL.

Table 5 Interaction analysis for 3-locus models by using logistic regression

rs507392	rs1617640	rs551238	OR (95% CI)*	P values
TT	TT	AA	1.00	–
TT	TT	AC or CC	1.05 (0.61–1.49)	0.627
TT	TG or GG	AA	1.09 (0.59–1.96)	0.574
TT	TG or GG	AC or CC	1.64 (0.81–3.33)	0.216
TC or CC	TT	AA	1.23 (1.14–2.02)	<0.001
TC or CC	TT	AC or CC	2.22 (1.16–4.17)	<0.001
TC or CC	TG or GG	AA	2.27 (1.05–4.76)	0.032
TC or CC	TG or GG	AC or CC	3.84 (1.75–8.33)	<0.001

*Adjusted for gender, age, smoke and alcohol status, duration of diabetes, SBP, DBP, BMI, WC, and HDL.

SNPs of EPO gene (GG genotype of rs1617640, and CC genotype of rs551238, CC genotype of rs507392) were associated with increased susceptibility of DR in Caucasian T2DM subjects, which were consistent with the results in the present study. However, Williams et al.²¹ conducted a meta-analysis and indicated little evidence for the association of the EPO promoter polymorphism, rs161740, with the combined phenotype of proliferative retinopathy and end-stage renal disease, and they presented evidence that several previously reported genetic associations with DN in type 1 diabetes could not be replicated in a large, homogeneous sample of subjects with type 1 diabetes. A recent study conducted by Hosseini et al.²² also suggested that rs1617640 (EPO) was not significantly associated with DR status, combined SDR-DN phenotype, time to SDR or time to DN, and provide suggestive collective evidence for association between DR and variants previously associated with DN without reaching statistical significance at any single locus. In another study conducted on a Caucasian T2DM cohort, the allele T in SNP rs1617640 was shown to be associated with an increased risk of PDR,¹⁵ and a major and important difference between the two cohorts is the complete lack of ESRD in the Abhary et al. study, compared with the majority of cases having ESRD in the Tong et al. study. A previous study in Chinese population by Song and colleagues¹⁶ carried out a similar investigation and reached a different result. A possible explanation for this difference was that the sample of the current study was larger than that in study by Song et al. Animal studies^{15,23} have shown that increased EPO concentrations in ischemic retinas and EPO inhibitors preventing neovascularization, further supporting the role of EPO in the development of PDR.²³

It was known that genetic susceptibility to DR was related to several genes; however, no study involved in

the interaction among many SNPs of EPO gene was conducted previously. In this study, we analyzed the interaction among three SNPs by using GMDR model, and found a significant interaction among rs507392, rs1617640, and rs551238, and that subjects with TC or CC-TG or GG-AC or CC genotype have the highest DR risk. EPO is an important cytokine which could stimulate migration, proliferation, and angiogenesis in vascular endothelial cells.^{24,25} EPO expression has been shown to be influenced by SNPs in EPO¹⁶ and is elevated in the subjects with PDR.^{10,13,26} EPO is considered as a biologically plausible candidate gene with potential to influence susceptibility to develop DR. Chen et al.²³ also suggested that the increase of intraocular synthesis of EPO that occurs in DR can be considered to be a compensatory mechanism for restoring the damage induced by DM rather than a pathogenic contributor. This study suggested a significant association between EPO gene variation and DR. In addition, although carriers of C allele (AA + AC) in rs551238 were not associated with DR, we still found a significant association with DR risk when accompanied with rs507392 and rs1617640. These findings indicate that a minor gene (even when its main effects are close to nil) can have a strong effect on DR, due to the presence of gene–gene interaction, which was consistent with a Chinese study.²⁷

The limitations of this study should be considered. First, the present sample size was relatively small, although the number of study participants met the requirement for analysis. Additional, larger sample studies should be conducted in the future. A slight deviation from HWE was observed in the control group, because the control subjects were also DM patients. Third, we did not examine the variability of biochemical markers in function of different EPO genotypes.

In conclusion, we found that there was a significant association between three SNPs of EPO and increased DR risk. DR risks were higher in the subjects with C allele of 507392, G allele of rs1617640 or C allele of rs551238 genotype. In addition, we also found a significant interaction among rs507392, rs1617640, and rs551238, and also that subjects with TC or CC-TG or GG-AC or CC genotype have the highest DR risk.

Authors' Contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript, SJ, FYY, CZ, and HYY conducted the experiments, and FYF wrote the manuscript.

ACKNOWLEDGEMENTS

The writing of this paper was supported by the Third Affiliated Hospital of southern Medical University. We thank all the partners and staff who helped us in the process of this study.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. *Arch Ophthalmol* 1984;**102**:527-32
- 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. *Diab Technol Ther* 2015;**17**:112-8
- Karlberg C, Falk C, Green A, Sjölie AK, Grauslund J. Proliferative retinopathy predicts nephropathy: a 25-year follow-up study of type 1 diabetic patients. *Acta Diabetol* 2012;**49**:263-8
- Hietala K, Forsblom C, Summanen P, Groop PH. Heritability of proliferative diabetic retinopathy. *Diabetes* 2008;**57**:2176-80
- Looker HC, Nelson RG, Chew E, Looker HC, Nelson RG, Chew E, Klein R, Klein BE, Knowler WC, Hanson RL. Genome-wide linkage analyses to identify Loci for diabetic retinopathy. *Diabetes* 2007;**56**:1160-6
- Ferrara N. Vascular endothelial growth factor. The trigger for neovascularization in the eye. *Lab Investigat; a journal of technical methods and pathology* 1995;**72**:615-8
- Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. *Microvasc Res* 2002;**64**:326-33
- Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ. Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biological Chem* 1996;**271**:736-41
- Hammes HP, Weiss A, Hess S, Araki N, Horiuchi S, Brownlee M, Preissner KT. Modification of vitronectin by advanced glycation alters functional properties in vitro and in the diabetic retina. *Lab Investigat; a journal of technical methods and pathology* 1996;**75**:325-38
- Watanabe D, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M, Suzuma I, Ohashi H, Ojima T, Murakami T, Kobayashi T, Masuda S, Nagao M, Yoshimura N, Takagi H. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. *N Engl J Med* 2005;**353**:782-92
- Grimm C, Wenzel A, Acar N, Keller S, Seeliger M, Gassmann M. Hypoxic preconditioning and erythropoietin protect retinal neurons from degeneration. *Adv Exp Med Biol* 2006;**588**:119-31
- Garcia-Ramirez M, Hernandez C, Simo R. Expression of erythropoietin and its receptor in the human retina: a comparative study of diabetic and non-diabetic subjects. *Diab Care* 2008;**31**:1189-94
- Takagi H, Watanabe D, Suzuma K, et al. Novel role of erythropoietin in proliferative diabetic retinopathy. *Diab Res Clin Pract* 2007;**77**:S62-4
- Abhary S, Burdon KP, Casson RJ, Goggin M, Petrovsky NP, Craig JE. Association between erythropoietin gene polymorphisms and diabetic retinopathy. *Archive Ophthalmol* 2010;**128**:102-6
- Tong Z, Yang Z, Patel S, Chen H, Gibbs D, Yang X, Hau VS, Kaminoh Y, Harmon J, Pearson E, Buehler J, Chen Y, Yu B, Tinkham NH, Zabriskie NA, Zeng J, Luo L, Sun JK, Prakash M, Hamam RN, Tonna S, Constantine R, Ronquillo CC, Sadda S, Avery RL, Brand JM, London N, Anduze AL, King GL, Bernstein PS, Watkins S, Jorde LB, Li DY, Aiello LP, Pollak MR, Zhang K. Promoter polymorphism of the erythropoietin gene in severe diabetic eye and kidney complications. *Proc Natl Acad Sci USA* 2008;**105**:6998-7003
- Song Q, Zhang Y, Wu Y, Zhou F, Qu Y. Association of erythropoietin gene polymorphisms with retinopathy in a Chinese cohort with type 2 diabetes mellitus. *Clin Exp Ophthalmol* 2015;**43**:544-9
- Balasubbu S, Sundaresan P, Rajendran A, Ramasamy K, Govindarajan G, Perumalsamy N, Hejtmancik JF. Association analysis of nine candidate gene polymorphisms in Indian patients with type 2 diabetic retinopathy. *BMC Med Genet* 2010;**11**:158
- Lohman T, Roche AF, Martorell R. *Standardization of anthropometric measurements*. Champaign, IL: Human Kinetics, 1988
- Alwakeel S, Al-Suwaid A, Isnani A, Al-Harbi A, Alam A. Concomitant macro and microvascular complications in diabetic nephropathy. *Saudi J Kidney Dis Transplant* 2009;**20**:402-9
- Lou XY, Chen GB, Yan L, Ma JZ, Zhu J, Elston RC, Li MD. A generalized combinatorial approach for detecting gene-by gene and gene-by-environment interactions with application to nicotine dependence. *Am J Hum Genet* 2007;**80**:1125-37
- Williams WW, Salem RM, McKnight AJ, Sandholm N, Forsblom C, Taylor A, Guiducci C, McAteer JB, McKay GJ, Isakova T, Brennan EP, Sadlier DM, Palmer C, Söderlund J, Fagerholm E, Harjutsalo V, Lithovius R, Gordin D, Hietala K, Kytö J, Parkkonen M, Rosengård-Bärlund M, Thorn L, Syreeni A, Tolonen N, Saraheimo M, Wadén J, Pitkaniemi J, Sarti C, Tuomilehto J, Tryggvason K, Österholm AM, He B, Bain S, Martin F, Godson C, Hirschhorn JN, Maxwell AP, Groop PH, Florez JC GENIE Consortium. Association testing of previously reported variants in a large case-control meta-analysis of diabetic nephropathy. *Diabetes* 2012;**61**:2187-94
- Hosseini SM, Boright AP, Sun L, Canty AJ, Bull SB, Klein BE, Klein R, Paterson AD DCCT/EDIC Research Group. The association of previously reported polymorphisms for microvascular complications in a meta-analysis of diabetic retinopathy. *Hum Genet* 2015;**134**:247-57
- Chen J, Connor KM, Aderman CM, Willett KL, Aspegren OP, Smith LE. Suppression of retinal neovascularization by erythropoietin siRNA in a mouse model of proliferative retinopathy. *Invest Ophthalmol Vis Sci* 2009;**50**:1329-35
- Yamaji R, Okada T, Moriya M, Naito M, Tsuruo T, Miyatake K, Nakano Y. Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. *Eur J Biochem* 1996;**239**:494-500
- Anagnostou A, Lee ES, Kessimian N, Levinson R, Steiner M. Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. *Proc Natl Acad Sci USA* 1990;**87**:5978-82

26. Katsura Y, Okano T, Matsuno K, Osako M, Kure M, Watanabe T, Iwaki Y, Noritake M, Kosano H, Nishigori H, Matsuoka T. Erythropoietin is highly elevated in vitreous fluid of patients with proliferative diabetic retinopathy. *Diab Care* 2005;**28**:2252-4
27. Luo W, Guo Z, Wu M, Hao C, Hu X, Zhou Z, Zhou Z, Yao X, Zhang L, Liu J. Association of peroxisome proliferator-activated receptor $\alpha/\delta/\gamma$ with obesity, and gene-gene interaction, in the Chinese Han population. *J Epidemiol* 2013;**23**:187-94

(Received September 9, 2015, Accepted March 9, 2016)