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ORIGINAL ARTICLE

Association of C(-106)T Polymorphism in Aldose Reductase Gene with Diabetic Retinopathy in Chinese Patients with Type 2 Diabetes Mellitus[△]

Yu Deng^{1,2}, Xiu-fen Yang², Hong Gu², Apiradee Lim³, Munkhtulga Ulziibat⁴, Torkel Snellingen⁴, Jun Xu², Kai Ma², and Ning-pu Liu^{2*}

¹Department of Ophthalmology, Fu Xing Hospital, Capital Medical University, Beijing 100038, China

²Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology and Visual Sciences Key Laboratory, Beijing 100730, China

³Department of Mathematics and Computer Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Pattani 94000, Thailand

⁴Sekwa Eye Hospital, Beijing 100088, China

Key words: diabetic retinopathy; aldose reductase gene; single nucleotide polymorphism

Objective To identify the possible association between C(-106)T polymorphism of the aldose reductase (ALR) gene and diabetic retinopathy (DR) in a cohort of Chinese patients with type 2 diabetes mellitus (T2DM).

Methods From November 2009 to September 2010, patients with T2DM were recruited and assigned to DR group or diabetic without retinopathy (DWR) group according to the duration of diabetes and the grading of 7-field fundus color photographs of both eyes. Genotypes of the C(-106)T polymorphism (rs759853) in ALR gene were analyzed using the MassARRAY genotyping system and an association study was performed.

Results A total of 268 T2DM patients (129 in the DR group and 139 in the DWR group) were included in this study. No statistically significant differences were observed between the 2 groups in the age of diabetes onset ($P=0.10$) and gender ($P=0.78$). The success rate of genotyping for the study subjects was 99.6% (267/268), with one case of failure in the DR group. The frequencies of the T allele in the C(-106)T polymorphism were 16.0% (41/256) in the DR group and 19.4% (54/278) in the DWR group ($P=0.36$). There was no significant difference in the C(-106)T genotypes between the 2 groups ($P=0.40$). Compared with the wild-type genotype, odds ratio (OR) for the risk of DR was 0.7 (95% CI, 0.38-1.3) for the heterozygous CT genotype and 0.76 (95% CI, 0.18-3.25) for the homozygous TT genotype. The risk of DR

was positively associated with microalbuminuria (OR=4.61; 95% CI, 2.34-9.05) and insulin therapy (OR=3.43; 95% CI, 1.94-6.09).

Conclusions Microalbuminuria and insulin therapy are associated with the risk of DR in Chinese patients with T2DM. C(-106)T polymorphism of the ALR gene may not be significantly associated with DR in Chinese patients with T2DM.

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DIABETIC retinopathy (DR) is the leading cause of legal blindness in working-age adults, accounting for 8% of the legal blindness in the United States¹ and in China.² In recent years, there has been considerable evidence suggesting that DR is influenced not only by the duration of diabetes and blood glucose level but also by genetic predisposition.³⁻⁷

Aldose reductase (ALR), the first and the rate-limiting enzyme in the polyol pathway, catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of glucose to sorbitol. Experimental studies suggest that sorbitol accumulate excessively in various tissues of the diabetic rats⁸ or in the rat glomerular mesangial cells cultured under high glucose conditions.⁹ Moreover, both increased enzyme activity and gene expression of ALR have been reported in patients with diabetic microvascular complications.¹⁰ ALR has thus been proposed to be an important factor in the pathogenesis of diabetic microangiopathy. The C(-106)T polymorphism located at the promoter of the ALR gene has been reported to be associated with DR in Caucasian and Asian patients with type 1 and 2 diabetes mellitus.¹¹⁻¹⁹ However, results of various studies regarding the relationship between DR and the C(-106)T polymorphism are conflicting and inconclusive. While several studies suggested an association between DR and the C(-106)T polymorphism,^{4,7,20,21} some other studies did not support this observation.^{22,23} In this study, we aimed to verify the possible association of the C(-106)T polymorphism of the ALR gene with the risk of DR with a cohort of Chinese patients with type 2 diabetes mellitus (T2DM).

SUBJECTS AND METHODS

Study participants and clinical evaluation

Between November 2009 and September 2010, patients with T2DM were recruited from the Desheng Community of downtown Beijing. Written informed consent was obtained from each participant before enrollment. Patients were enrolled into the study if: (1) they reported physician-diagnosed T2DM treated with insulin, oral hypoglycemic agents or healthy diet only; (2) or had a fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dl) in at least two previous

examinations or a random plasma glucose ≥ 11.1 mmol/L (200 mg/dL). All the participants underwent a standardized evaluation consisting of a questionnaire assessing demographic information and medical history, anthropometric examinations including body mass index (BMI) and waist-hip ratio, and a comprehensive ophthalmological examination including best corrected visual acuity, slit lamp biomicroscopy, fundus examination, and fundus photography. Seven-field 30-degree stereoscopic fundus color photographs of optic disc and macula were taken through dilated pupils for all the patients using a digital fundus camera (Zeiss, Oberkochen, Germany). Fasting blood samples (15 mL) were collected to measure the fasting plasma glucose, glycosylated hemoglobin A1c (HbA1c), creatinine, uric acid, and lipid profiles [levels of total cholesterol, triglycerides, high-density and low-density lipoprotein cholesterol (HDL-C and LDL-C)]. Fasting urine samples were obtained to detect microalbuminuria.

Two ophthalmologists independently graded the fundus photographs and the final diagnosis for each patient was determined based on the higher grading of the worse eye.²⁴ The duration of diabetes was defined as the interval between the initial diagnosis by a physician and the time of enrollment into the present study. Based on the duration of diabetes and the grading of fundus photographs, patients were assigned into the diabetic without retinopathy (DWR) group if they had a duration of T2DM ≥ 10 years with no signs of DR (microaneurysms, hemorrhages or exudates) or if they had a duration of T2DM ≥ 15 years with fewer than 5 microaneurysms.^{4,25} Patients with 5 or more microaneurysms in at least one eye were assigned into the DR group.²⁵ Patients who did not meet the criteria of either DWR or DR were excluded from the study.

The Ethics Committee of Beijing Tongren Hospital approved the study protocol and the procedures were performed in accordance with the Declaration of Helsinki.

Genotyping for ALR polymorphism

Blood samples (2 mL each) were collected from all participants and stored at -80°C before DNA extraction. Genomic DNA was isolated from venous blood leukocytes using a genomic DNA extraction and purification kit (TIANamp Swab DNA Kit, Tiangen Biotech, Beijing, China).

The C(-106)T polymorphism (rs759853) in the promoter region of ALR gene was genotyped using the Sequenom MassARRAY technology (Sequenom MassARRAY, Bioyong Technologies Inc., Beijing, China).^{26, 27}

Statistical analysis

Statistical analysis was performed with the R statistical analysis packages. Baseline characteristics of the DR and DWR groups were compared using *t*-test for continuous variables or Chi-square test for categorical variables. Results are expressed as means $\pm$ SD or median (range). Genotype frequencies of the ALR C(-106)T polymorphism were checked for Hardy-Weinberg equilibrium using Chi-square test. Chi-square test was also used to analyze the distribution of genotypes and alleles. Associations between the ALR C(-106)T polymorphism and the presence of DR were tested by calculating the odds ratio (OR) and the 95% confidence interval (CI) using multivariate logistic regression. $P < 0.05$ was considered statistically significant.

The power to detect a significant association between the T allele of the ALR C(-106)T polymorphism and DR was calculated under the condition of different hypothetical OR values. To detect a significantly protective effect of the T allele, the sample size in this study had 82.4% power if the T allele carriers had an OR of 0.5 and 30.4% power if the T allele carriers had an OR of 0.7. To detect a significant risk

effect of the T allele, the sample size in this study had 81.7% power if the T allele carriers had an OR of 2.0 and 37.6% power if the T allele carriers had an OR of 1.5.

RESULTS

A total of 268 patients, including 129 with DR and 139 with DWR, were enrolled in this study. Clinical features of the patients are displayed in Table 1. The mean age of diabetic onset was 48.68 ± 9.58 years in the DR group and 50.45 ± 8.00 years in the DWR group ($P=0.10$). Percentage of males was 44.2% in the DR group and 41.7% in the DWR group ($P=0.78$). Compared with the DWR group, the DR group had higher levels of creatinine ($P=0.02$), fasting plasma glucose ($P=0.01$), HbA1c ($P<0.001$), and higher incidence of microalbuminuria ($P<0.001$). The DR group also had a higher percentage of patients taking insulin therapy compared with the DWR group (62.8% vs. 33.8%, $P<0.001$). No statistically significant difference was observed between the DR and the DWR groups in the duration of diabetes ($P=0.25$), BMI ($P=0.29$), blood pressure (systolic blood pressure, $P=0.58$; diastolic blood pressure, $P=0.34$), serum levels of uric acid ($P=0.84$), and lipid profiles (total cholesterol, $P=0.70$; triglycerides, $P=0.75$; HDL-C, $P=0.98$; LDL-C, $P=0.98$) (Table 1).

Table 1. Clinical characteristics of the 268 patients with type 2 diabetes mellitus[§]

Group	Sex [male (%)]	Age of onset ($\bar{x} \pm s$, year)	Duration of diabetes ($\bar{x} \pm s$, year)	BMI ($\bar{x} \pm s$, kg/m ²)	Microalbumi- nuria [<i>n</i> (%)]	Systolic blood pressure ($\bar{x} \pm s$, mmHg)	Diastolic blood pressure ($\bar{x} \pm s$, mmHg)	Insulin therapy [<i>n</i> (%)]
DWR (<i>n</i> =139)	58 (41.7)	50.45±8.00	15.06±4.38	24.55±4.00	17 (12.2)	136.00±16.97	77.38± 8.82	47 (33.8)
DR (<i>n</i> =129)	57 (44.2)	48.68±9.58	14.59±7.52	24.99±3.78	48 (37.2)	137.10±16.20	78.92±10.05	81 (62.8)
<i>P</i>	0.78	0.10	0.25	0.29	<0.001	0.58	0.34	<0.001

Group	HbA1c ($\bar{x} \pm s$, %)	FBG ($\bar{x} \pm s$, mmol/L)	Creatinine ($\bar{x} \pm s$, μmol/L)	UA ($\bar{x} \pm s$, μmol/L)	TC ($\bar{x} \pm s$, mmol/L)	TG ($\bar{x} \pm s$, mmol/L)	HDL-C ($\bar{x} \pm s$, mmol/L)	LDL-C ($\bar{x} \pm s$, mmol/L)	CRP ($\bar{x} \pm s$, mg/L)
DWR (<i>n</i> =139)	7.07±1.40	8.23±2.46	65.14±16.50	275.80±77.83	5.22±0.96	1.68±1.09	1.21±0.29	3.08±0.79	0.25±0.33
DR (<i>n</i> =129)	7.93±1.67	9.20±2.96	71.49±24.42	277.70±68.38	5.27±1.13	1.67±1.17	1.23±0.32	3.08±0.86	0.24±0.32
<i>P</i>	<0.001	0.01	0.02	0.84	0.70	0.75	0.98	0.98	0.84

§: Plus-minus values are means $\pm$ SD.

Differences between the diabetic retinopathy (DR) and the diabetic without retinopathy (DWR) groups were compared using *t*-test or Chi-square test.

BMI: body mass index; HbA1c, glycosylated hemoglobin A1c; FBG: fasting blood glucose; UA: uric acid; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein.

The genotype and allele frequencies of the ALR C(-106)T polymorphism in the DR and DWR groups are listed in Table 2. The success rate of genotyping for the patients was 99.6% (267/268). Frequencies of the ALR C(-106)T genotypes were under Hardy-Weinberg equilibrium in the DR ($P=0.20$) and the DWR group ($P=1.00$). The frequency of the minor T allele was 16.0% (41/256) in the DR group and 19.4% (54/278) in the DWR group ($P=0.36$). No significant difference in genotype distribution was observed between the DR and DWR groups ($P=0.40$). Compared with the wild type CC genotype, the OR for the risk of DR was 0.7 (95% CI: 0.38-1.3) for the heterozygous CT genotype and 0.76 (95% CI: 0.18-3.25) for the homozygous TT genotype after adjustment for the age of diabetic onset, duration of diabetes, BMI, HbA1c, fasting plasma glucose, creatinine level, microalbuminuria, and insulin therapy.

Significant positive association of DR was observed with microalbuminuria (adjusted OR=4.61, 95% CI: 2.34-9.05; $P<0.001$) and insulin therapy (adjusted OR=3.43, 95% CI: 1.94-6.09; $P<0.001$) (Table 3).

DISCUSSION

In this study, we investigated the association of ALR C(-106)T polymorphism with susceptibility to DR. Consistent with studies in Brazilian,²³ French,²⁸ and Australian patients²⁰ with type 1 diabetes, and Euro-Brazilian patients with type 2 diabetes,²² the present study found no association between the ALR C(-106)T polymorphism and the risk of DR in this Chinese cohort of T2DM. However, a Japanese study¹¹ and a Chilean study²¹ reported a higher risk of DR with the CC genotype of the C(-106)T polymorphism as compared with the CT or TT genotype. A previous study on Chinese patients with T2DM also reported a higher risk of DR with the CC genotype of the C(-106)T polymorphism.⁷ The discrepancy among different studies could be due to the different genetic background, sampling or experimental bias, or the presence of confounding factors. Indeed, the CC genotype frequency of the C(-106)T polymorphism has been reported to be higher in Asian^{29,30} than in Caucasian^{20,23,31} and Chilean populations.²¹ Moreover, the relatively small sample size in

Table 2. Frequencies of the C(-106)T polymorphism genotypes and alleles in the ALR gene

Group	Genotype			H-W p	Allele	
	CC	CT	TT		C	T
DWR (n=139)	90(64.7)	44(31.7)	5(3.6)	1	224(80.6)	54(19.4)
DR (n=128)*	92(71.9)	31(24.2)	5(3.9)	0.20	215(84.0)	41(16.0)
P		0.40				0.36
Adjusted OR (95% CI)	1	0.70 (0.38,1.30)	0.76 (0.18,3.25)		1	1.26 (0.79,2.03)
P (Wald's test)		0.26	0.71			0.30

ALR: aldose reductase; *one case in the DR group was not successfully genotyped, therefore only 128 cases were analyzed in the frequencies of C(-106)T genotypes and alleles.

Genotype distributions for single nucleotide polymorphism were in Hardy-Weinberg equilibrium. Multiple logistic regression analysis was used to calculate odds ratio (OR) and 95% confidence interval (CI) by comparing the DR group and the DWR group after adjusting for the age of diabetic onset.

Table 3. Clinical characteristics and the risk of DR by multivariate logistic regression

Clinical characteristic	Adjusted OR (95%CI)	P (Wald's test)
Age of diabetic onset (years)	0.97 (0.93, 1)	0.072
Duration of diabetes (years)	0.94 (0.89, 0.99)	0.019
BMI (kg/m ²)	1.08 (0.61, 1.89)	0.800
Microalbuminuria (+/-)	4.61 (2.34, 9.05)	< 0.001
Insulin therapy (yes/no)	3.43 (1.94, 6.09)	< 0.001
HbA1c (%)	1.94 (0.82, 4.62)	0.130
Fasting plasma glucose (mmol/L)	1.46 (0.61, 3.45)	0.400
Creatinine (μmol/L)	1.09 (0.27, 4.49)	0.910

the Chilean study (53 subjects)²¹ or the previous study with Chinese T2DM patients (40 with DR and 105 with DWR)⁷ could potentially lead to a selection bias. In addition, many environmental and genetic factors could contribute to the pathogenesis of DR, which could bias the results. Other factors possibly interfering with the study outcomes include the diagnostic criteria of DR and DWR, which are not the same in different studies, and the recruitment methods of the study participants. For example, most studies recruited participants from hospitals whereas some studies employed population- or community-based recruitment procedures. It should be noted that the sample size in the present study had sufficient statistical power to detect only a strong association between the ALR C(-106)T polymorphism and DR. We therefore cannot exclude the possibility of a weak association between the ALR C(-106)T polymorphism and DR in Chinese population.

Poor glycemic control and higher glycosylated hemoglobin levels are the conventional risk factors for the development or progression of DR.³¹⁻³⁴ In the present study, we confirmed that these two factors are associated with the risk of DR. Consistent with previous findings,^{35, 36} we also found that the use of insulin was associated with a higher risk of DR. One possible explanation is that patients using insulin are more likely to have a poor glycemic control or to be at the late stage of the disease.³⁷ Poor glycemic control with oral agents is usually the primary reason to begin insulin treatment. The patients with insulin use are therefore at a higher risk of having DR. In the present study, 37.2% of the patients in the DR group had microalbuminuria. The association between DR and microalbuminuria observed in the present study may be explained by the hypothesis that microalbuminuria might represent a state of generalized vascular dysfunction.³⁸ Furthermore, microalbuminuria and DR may share common determinants.

In conclusion, we confirmed that patients with DR have a higher percentage of microalbuminuria ($P<0.001$) and are more likely to use insulin ($P<0.001$) compared with DWR patients. However, no evidence was observed to support a significant association of the C(-106)T polymorphism with DR in this study, suggesting that the C(-106)T polymorphism of the ALR gene might not be a major susceptibility factor of DR in Chinese patients with T2DM. Whether there is a weak association between the ALR C(-106)T polymorphism and DR in Chinese population needs to be confirmed with a larger-size sample in future studies.

REFERENCES

1. Congdon NG, Friedman DS, Lietman T. Important causes of visual impairment in the world today. *JAMA* 2003; 290: 2057-60.
2. Xie X, Xu L, Yang H, et al. Frequency of diabetic retinopathy in the adult population in China: the Beijing Eye Study 2001. *Int Ophthalmol* 2009; 29: 485-93.
3. Hanis CL, Hallman D. Genetics of diabetic retinopathy. *Curr Diab Rep* 2006; 6: 155-61.
4. Demaine A, Cross D, Millward A. Polymorphisms of the aldose reductase gene and susceptibility to retinopathy in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2000; 41: 4064-8.
5. Arar NH, Freedman BI, Adler SG, et al. Heritability of the severity of diabetic retinopathy: the find-eye study. *Invest Ophthalmol Vis Sci* 2008; 49: 3839-5.
6. Olmos P, Futers S, Acosta AM, et al. (AC)23 [Z-2] polymorphism of the aldose reductase gene and fast progression of retinopathy in Chilean type 2 diabetics. *Diabetes Res Clin Pract* 2000; 47: 169-76.
7. Li Q, Xie P, Huang J, et al. Polymorphisms and functions of the aldose reductase gene 5' regulatory region in Chinese patients with type 2 diabetes mellitus. *Chin Med J (Engl)* 2002; 115: 209-13.
8. Greene DA, Lattimer SA, Sima AA. Sorbitol, phosphoinositides, and sodium-potassium-atpase in the pathogenesis of diabetic complications. *N Engl J Med* 1987; 316: 599-606.
9. Lewko B, Latawiec E, Maryn A, et al. Osmolarity and glucose differentially regulate aldose reductase activity in cultured mouse podocytes. *Exp Diabetes Res* 2011; 278963.
10. Abhary S, Burdon KP, Laurie KJ, et al. Aldose reductase gene polymorphisms and diabetic retinopathy susceptibility. *Diabetes Care* 2010; 33: 1834-6.
11. Katakami N, Kaneto H, Takahara M, et al. Aldose reductase c-106t gene polymorphism is associated with diabetic retinopathy in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract* 2011; 92: e57-60.
12. Ng DPK. Human genetics of diabetic retinopathy: current perspectives. *J Ophthalmol* 2010 [cited 2012 Jul 13]. Available from: <http://www.hindawi.com/journals/joph/2010/172593/>
13. Moczulski DK, Scott L, Antonellis A, et al. Aldose reductase gene polymorphisms and susceptibility to diabetic nephropathy in type 1 diabetes mellitus. *Diabet Med* 2000; 17: 111-8.
14. Neamat-Allah M, Feeney SA, Savage DA, et al. Analysis of the association between diabetic nephropathy and polymorphisms in the aldose reductase gene in type 1 and type 2 diabetes mellitus. *Diabet Med* 2001; 18: 906-14.
15. Wang Y, Ng MC, Lee SC, et al. Phenotypic heterogeneity

- and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. *Diabetes Care* 2003; 26: 2410-5.
16. Makiishi T, Araki S, Koya D, et al. C-106T polymorphism of AKR1B1 is associated with diabetic nephropathy and erythrocyte aldose reductase content in Japanese subjects with type 2 diabetes mellitus. *Am J Kidney Dis* 2003; 42: 943-51.
 17. Sivenius K, Niskanen L, Voutilainen-Kaunisto R, et al. Aldose reductase gene polymorphisms and susceptibility to microvascular complications in type 2 diabetes. *Diabet Med* 2004; 21: 1325-33.
 18. Sivenius K, Pihlajamäki J, Partanen J, et al. Aldose reductase gene polymorphisms and peripheral nerve function in patients with type 2 diabetes. *Diabetes Care* 2004; 27: 2021-6.
 19. Gosek K, Moczulski D, Zukowska-Szczechowska E, et al. C-106t polymorphism in promoter of aldose reductase gene is a risk factor for diabetic nephropathy in type 2 diabetes patients with poor glycaemic control. *Nephron Exp Nephrol* 2005; 99: e63-7.
 20. Kao YL, Donaghue K, Chan A, et al. A novel polymorphism in the aldose reductase gene promoter region is strongly associated with diabetic retinopathy in adolescents with type 1 diabetes. *Diabetes* 1999; 48: 1338-40.
 21. Olmos P, Bastías MJ, Vollrath V, et al. C(-106)T polymorphism of the aldose reductase gene and the progression rate of diabetic retinopathy. *Diabetes Res Clin Pract* 2006; 74: 175-82.
 22. Santos KG, Tschiedel B, Schneider J, et al. Diabetic retinopathy in Euro-Brazilian type 2 diabetic patients: relationship with polymorphisms in the aldose reductase, the plasminogen activator inhibitor-1 and the methylene-tetrahydrofolate reductase genes. *Diabetes Res Clin Pract* 2003; 61: 133-6.
 23. Richeti F, Noronha RM, Waetge RT, et al. Evaluation of AC(n) and C(-106)T polymorphisms of the aldose reductase gene in Brazilian patients with dm1 and susceptibility to diabetic retinopathy. *Mol Vis* 2007; 13: 740-5.
 24. Chaturvedi N, Sjoelie AK, Svensson A, et al. The Diabetic Retinopathy Candesartan Trials (DIRECT) Programme, rationale and study design. *J Renin Angiotensin Aldosterone Syst* 2002; 3: 255-61.
 25. Suganthalakshmi B, Anand R, Kim R, et al. Association of VEGF and eNOS gene polymorphisms in type 2 diabetic retinopathy. *Mol Vis* 2006; 12: 336-41.
 26. Abel K, Reneland R, Kammerer S, et al. Genome-wide snp association: identification of susceptibility alleles for osteoarthritis. *Autoimmun Rev* 2006; 5: 258-63.
 27. Liu S, Chen HD, Makarevitch I, et al. High-throughput genetic mapping of mutants *via* quantitative single nucleotide polymorphism typing. *Genetics* 2010; 184: 19-26.
 28. Fanelli A, Hadjadj S, Gallois Y, et al. Polymorphism of aldose reductase gene and susceptibility to retinopathy and nephropathy in caucasians with type 1 diabetes. *Arch Mal Coeur Vaiss* 2002; 95: 701-8.
 29. Watarai A, Nakashima E, Hamada Y, et al. Aldose reductase gene is associated with diabetic macroangiopathy in Japanese type 2 diabetic patients. *Diabet Med* 2006; 23: 894-9.
 30. So WY, Wang Y, Ng MC, et al. Aldose reductase genotypes and cardiorenal complications: an 8-year prospective analysis of 1,074 type 2 diabetic patients. *Diabetes Care* 2008; 31: 2148-53.
 31. dos Santos KG, Canani LH, Gross JL, et al. The -106CC genotype of the aldose reductase gene is associated with an increased risk of proliferative diabetic retinopathy in Caucasian-Brazilians with type 2 diabetes. *Mol Genet Metab* 2006; 88: 280-4.
 32. Looker HC, Krakoff J, Knowler WC, et al. Longitudinal studies of incidence and progression of diabetic retinopathy assessed by retinal photography in pima indians. *Diabetes Care* 2003; 26: 320-6.
 33. Stratton IM, Kohner EM, Aldington SJ, et al. UKPDS 50: risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis. *Diabetologia* 2001; 44: 156-63.
 34. van Leiden HA, Dekker JM, Moll AC, et al. Risk factors for incident retinopathy in a diabetic and nondiabetic population: the Hoorn study. *Arch Ophthalmol* 2003; 121: 245-51.
 35. Chorny A, Lifshits T, Kratz A, et al. Prevalence and risk factors for diabetic retinopathy in type 2 diabetes patients in Jewish and Bedouin populations in southern Israel. *Harefuah* 2011; 150: 906-10, 935.
 36. Thomas RL, Dunstan F, Luzio SD, et al. Incidence of diabetic retinopathy in people with type 2 diabetes mellitus attending the Diabetic Retinopathy Screening Service for wales: retrospective analysis. *BMJ* 2012; 344: e874.
 37. U.K. Prospective Diabetes Study Group. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. *Diabetes* 1995; 44: 1249-58.
 38. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, et al. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 1989; 32: 219-26.