Association between diabetic retinopathy and genetic variations in α2β1 integrin, a platelet receptor for collagen

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Platelets might be involved in the pathogenesis of diabetic microangiopathy. Wide interindividual variations in the density of a platelet collagen receptor (α2β1 integrin or glycoprotein Ia/IIa) are reportedly associated with polymorphism(s) in the gene encoding the α subunit of the receptor, including a Bgl II polymorphism in intron 7. The aim of the present study was to determine the relationship between the Bgl II polymorphism and the susceptibility to diabetic microangiopathy. A case-control study comparing 227 patients with type II diabetes mellitus (119 with versus 108 without diabetic retinopathy) as well as 169 nondiabetic subjects demonstrated that genotypes with Bgl II (+) allele had a significant increase in the risk for retinopathy. The odds ratio for Bgl II (+/+) to Bgl II (−/−) was 3.41 (95% CI, 1.49-7.78, *P* = .0036) when analysis was confined to those with a disease duration of diabetes of 10 years or more. The present study suggests that the presence of a Bg II (+) allele is a genetic risk factor for diabetic retinopathy. (Blood. 2000;95: 1560-1564)

Introduction

The platelet membrane glycoprotein (GP) Ia/IIa, α2β1 integrin, serves as a platelet receptor for collagen.[1-4] It mediates platelet primary adhesion to subendothelial tissues, which is an essential first step in thrombus formation. The gene encoding α2 integrin has at least 8 polymorphisms, including 2 silent polymorphisms located within the I domain,[5] ^{224}Phe (TTT/TTC) due to a T/C transition at nucleotide 807 (807T/C) and ^{246}Thr (ACA/ACG) due to an A/G transition at nucleotide 873 (873A/G) (numbers according to Takada et al[6]), and a Bgl II restriction fragment length polymorphism (Bgl II, +/−) within intron 7.[7] These 3 polymorphisms are in linkage disequilibrium, the Bgl II (+) allele being linked to the 807T allele and 873A allele and the Bgl II (−) allele being linked to the 807C allele and 873G allele.[7] It was reported that the platelet α2β1 density and the extent of platelet adhesion to collagen were higher in individuals with the 807T-(873A- or Bgl II (+)-) homozygote than in individuals with the 807C-(873G- or Bgl II (−)-) homozygote.[7,8] Frequency of the 807T allele was reported to be 33.6%, 31.4%, and 53.9% in the healthy Caucasians, African Americans, and Native Americans, respectively.[9] Relationships between these polymorphisms and the prevalence of myocardial infarction or stroke have been reported, and the 807T allele has been shown to be at risk.[10-12]

Platelets from diabetic patients are hyperreactive to aggregating agents, such as adenosine diphosphate, collagen, and thrombin. There is increased secretion of β-thromboglobulin and platelet factor 4, which are markers of platelet activation in vivo.[13-15] The values of platelet activation markers in diabetic patients without microangiopathy are higher than those in patients without microangiopathy.[16,17] Also, the importance of platelets for the development of diabetic retinopathy or nephropathy is supported by several studies indicating the beneficial effects of antiplatelet therapy.[13,18-21] Thus, platelets are thought to be involved in the development of diabetic retinopathy or nephropathy, although the mechanisms underlying the association between platelet functions and microangiopathies are incompletely known.[15]

For the development of diabetic retinopathy and nephropathy, disease duration of diabetes and glycemic control are major determinants. Some diabetic patients, however, do not develop these complications, even after a long duration of diabetes.[13] Familial clustering of diabetic retinopathy[22] and nephropathy[23] has been demonstrated. These observations suggest that some modulating factors for the risk of diabetic retinopathy or nephropathy are genetically transmitted.

To examine a hypothesis that a genetic variation on α2β1 integrin is associated with the development of diabetic microangiopathy, we analyzed the association between the Bgl II polymorphism and the susceptibility to retinopathy or nephropathy among patients with type II diabetes mellitus. We also investigated genotype distributions of the PLA1/A2 polymorphism[24] of integrin αIIbβ3 (GP IIb/IIIa), a platelet membrane receptor for fibrinogen and von Willebrand factor.

Patients and methods

Study subjects

Informed consent was obtained from all subjects enrolled into the study. Normal subjects (n = 169; 135 males, 34 females), recruited at Hibiya Medical Center (Tokyo, Japan), Fuji Electronics (Tokyo, Japan), and Keio University Hospital (Tokyo, Japan) for their regular checkups, were analyzed. The mean age was 47.1 ± 5.9 years (mean ± SD). They had no

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clinical or laboratory evidence of either past vascular disorders or any form of diabetes.

Patients were genetically unrelated 227 Japanese subjects who had had a diagnosis of type II diabetes mellitus, as defined by World Health Organization criteria,[25] and were followed up on a regular basis at an outpatient clinic of Saitama Social Insurance Hospital (Saitama, Japan). They were divided into 2 groups (Table 1). The first group included 119 patients with retinopathy (35 patients with retinopathy without nephropathy, group B; and 84 patients with both retinopathy and nephropathy, group C). The second group consisted of 108 patients without retinopathy or nephropathy (group A). The group A patients were selected to match groups B and C patients in terms of age at diagnosis of diabetes, sex, and disease duration after diagnosis of diabetes. Diabetic retinopathy was diagnosed by independent diabetic ophthalmologists using standard fundus photos and was classified as simple, preproliferative, and proliferative (either treated with photocoagulation or not). Diabetic nephropathy was diagnosed using the following criteria: (1) urinary excretion of albumin per gram (urinary albumin index; UAI) $\geq$ 30 mg/g and (2) the presence of retinopathy, which indicates diabetic microangiopathy. It was classified as microalbuminuria (UAI, 30-299 mg/g), overt proteinuria (UAI $\geq$ 300 mg/g), and chronic renal failure. Diabetic patients with UAI $\leq$ 20 mg/g were classified as ''no nephropathy.'' Those with UAI between 21 and 29 mg/g were assumed to be in the ''gray zone'' and were excluded from the study. Those with UAI $\geq$ 30 mg/g but without retinopathy were also excluded from the study because proteinuria is observed in diabetic patients with reasons other than diabetic nephropathy.[26] Clinical data, including age at diagnosis of diabetes, disease duration after diagnosis of diabetes, body mass index (BMI) at diagnosis of diabetes, hemoglobin (Hb) A_{1C}, antihypertensive drug treatment, and hyperlipidemia were collected from medical records of the patients.

Genotyping of Bgl II polymorphism and PLA1/A2 polymorphism

Genomic DNA was isolated from peripheral blood leukocytes in 227 patients with type II diabetes mellitus and 169 normal subjects, as described previously.[27] For genotyping of the Bgl II polymorphism, a 600-bp DNA fragment that contains the Bgl II (+/−) polymorphism was amplified using polymerase chain reaction (PCR) with a DNA thermal cycler (Perkin Elmer, Takara Biomedicals, Chiba, Japan). Briefly, the reaction was performed in a final volume of 100 µL containing 1 µg genomic DNA, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.2 mM each of deoxynucleotide triphosphate, and 2.5 U Taq polymerase.

The 2 oligonucleotide primers[7] were 5′-GATTTAACTTTCCCGACTGCCTTC (nucleotide number 2789-2812) and 5′-CATAGGTTTTTGGGGAACAGGTGG (nucleotide number 3346-3369). PCR conditions of 34 cycles in this study were as follows: the first 2 cycles were 94°C for 1 minute, 69°C for 1 minute, and 72°C for 1 minute; the second 2 cycles were 94°C for 1 minute, 67°C for 1 minute, and 72°C for 1 minute; the remaining 30 cycles were 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute. Amplified DNA was digested with a restriction enzyme Bgl II (Takara Shuzo, Osaka, Japan) at 37°C for 3 hours, followed by an electrophoresis on a 2% agarose gel. The PCR products containing Bgl II (+) would be cut into visible fragments of 200 bp and 400 bp, whereas those containing Bgl II (−) would not be cut.

Genotyping of the PLA1/A2 polymorphism was performed essentially as described.[24]

Statistics

Student's *t* test was used to compare age at diagnosis of diabetes, disease duration after diagnosis of diabetes, BMI at diagnosis of diabetes, and HbA_{1C} at diagnosis of diabetes between subgroups of patients. Analyses of genotype frequency counts were performed using the chi-square test. Multiple logistic regression analysis was performed to evaluate the relationship between those with versus those without retinopathy (categorical variable, yes or no) and other variables. Independent variables included in the analysis were Bgl II genotype (categorical variable, +/+, +/−, −/−), sex (categorical variable, male or female), age at diagnosis of diabetes (quantitative variable), disease duration from diagnosis of diabetes (quantitative variable), and BMI at diagnosis of diabetes (quantitative variable). Analysis of variance (ANOVA) was used for the comparison of the mean values of current HbA_{1C} levels among the 3 Bgl II genotypes. All statistical analyses were performed using Statview (version 5.0, for Macintosh, Abacus Concepts, Berkeley, CA). Variability in sampling associated with the estimated odds ratio (OR) was assessed by 2-sided 95% confidence intervals (CI). An OR (95% CI) greater than 1 was considered to be significant. A *P* value less than 0.05 was considered to be statistically significant.

Table 1. Characteristics of patients with type II diabetes mellitus

	Total	Without Retinopathy or Nephropathy (Group A)	With Retinopathy or Nephropathy (Group B + C)*	*P* Value†
No.	227	108	119	
Male sex (%)	43.2	46.3	40.3	.3652
Age at diagnosis of diabetes (y)‡	47.4 ± 9.8	47.6 ± 8.8	47.2 ± 10.7	.7681
Disease duration after the diagnosis (y)‡	17.7 ± 7.4	17.0 ± 5.2	18.3 ± 8.9	.1862
Hb_{A1C} at diagnosis of diabetes (%)‡	9.9 ± 2.7	9.4 ± 3.0	10.2 ± 2.5	.2321
BMI at diagnosis of diabetes (kg/m^2)‡	23.2 ± 3.7	23.1 ± 3.2	23.3 ± 4.2	.7654

*Group B consists of 35 patients with retinopathy without nephropathy. Group C consists of 84 patients with both retinopathy and nephropathy.

†Patients without retinopathy or nephropathy (Group A) versus patients with retinopathy or nephropathy (group B + C).

‡Values are mean ± SD.

BMI indicates body mass index.

Results

Bgl II polymorphism and susceptibility to diabetic retinopathy or nephropathy

To explore the clinical significance of the Bgl II polymorphism, we performed a cross-sectional study comparing diabetic patients with (n = 119) and without (n = 108 retinopathy. Genotyping was performed also on 169 nondiabetic subjects. The clinical characteristics of 227 patients are shown in Table 1. The 2 groups were well matched with regard to gender distribution, age at diagnosis of diabetes, disease duration after diagnosis of diabetes, HbA_{1C} at diagnosis of diabetes, and BMI at diagnosis of diabetes.

Table 2 shows Bgl II genotype distribution in patients and normal subjects. The genotype distribution in each group shown in Table 2 was in Hardy-Weinberg equilibrium. The frequency of the Bgl II (+/+, +/−) genotype in group B plus C patients (69.7%) was significantly higher than that in group A patients (55.6%, OR [95% CI] = 1.84 [1.07-3.16], *P* = .0270). The frequency of Bgl II (+/+, +/−) genotype in group C patients (71.4%) was significantly higher than in group A plus B patients (58.0%, OR = 1.81 [1.02-3.22], *P* = .0437), or in group A patients (55.6%, OR = 2.00 [1.10-3.65], *P* = .0242). When the analysis was confined to those patients with a disease duration of 10 years or more, the frequency of Bgl II (+/+, +/−) genotype was 72.4% for group B plus C patients, 55.1% for group A patients, 73.9% for group C patients, and 58.1% for group A plus B patients (OR = 2.14 [1.20-3.82], *P* = .0102, group B plus C versus group A patients; OR = 2.04 [1.06-3.94], *P* = .0262, group C versus group A plus B patients; OR = 2.31 [1.20-4.44], *P* = .0120, group C versus

Table 2. Genotype distribution of α2 integrin Bgl II polymorphism in patients with type II diabetes and in normal subjects

	n	Bgl II (+/+)/Bgl II (+/−) n (%)/n (%)	Bgl II (−/−) n (%)	*P* Value* and Odds Ratio* (OR)
All patients				
Without retinopathy or nephropathy (group A)	108	13 (12.0)/47 (43.6)	48 (44.4)	.0270 (OR = 1.84)
With retinopathy (group B + C)	119	24 (20.2)/59 (49.5)	36 (30.3)	.0242 (OR = 2.00)
Without nephropathy (group A + B)	143	21 (14.7)/62 (43.3)	60 (42.0)	.0437 (OR = 1.81)
With nephropathy (group C)	84	16 (19.0)/44 (52.4)	24 (28.6)	
Disease duration ≥10 y				
Without retinopathy or nephropathy (group A)	107	12 (11.2)/47 (43.9)	48 (44.9)	.0102 (OR = 2.14)
With retinopathy (group B + C)	98	23 (23.5)/48 (48.9)	27 (27.6)	.0120 (OR = 2.31)
Without nephropathy (group A + B)	136	20 (14.7)/59 (43.4)	57 (41.9)	.0262 (OR = 2.04)
With nephropathy (group C)	69	15 (21.7)/36 (52.2)	18 (26.1)	
Normal subjects	169	30 (17.8)/81 (47.9)	58 (34.3)	

*Bgl II (+/+) + Bgl II (+/−) versus Bgl II (−/−).

group A patients). These observations suggest that patients with the Bgl II (+/+, +/−) genotype have an increased risk for diabetic retinopathy or nephropathy. For normal subjects, the frequency of the Bgl II (+/+, +/−) genotype was 65.7% and the frequency of the Bgl II (−/−) genotype was 34.3%.

Next, the dose effect of the Bgl II (+) allele on the risk for retinopathy or nephropathy was analyzed in diabetic patients with a disease duration of 10 years or more. It was shown that the greater the number of the Bgl II (+) allele, the greater the risk for diabetic retinopathy or nephropathy. For retinopathy, the frequencies of Bgl II (+/+) genotype were 23.5% and 11.2% for group B plus C and group A patients, respectively, and the frequencies of Bgl II (+/−) genotype were 48.9% and 43.9% for group B plus C and group A patients, respectively. The ORs (95% CI) to Bgl II (−/−) were 1.82 (0.98-3.38, $P = .0580$) and 3.41 (1.49-7.78, $P = .0036$) for Bgl II (+/−) and Bgl II (+/+) genotypes, respectively (see Table 2). For nephropathy, the frequencies of Bgl II (+/+) genotype were 21.7% and 14.7% for group C and group A plus B patients, respectively, and the frequencies of Bgl II (+/−) genotype were 52.2% and 43.4% for group C and group A plus B patients, respectively. The ORs (95% CI) to Bgl II (−/−) were 1.93 (0.99-3.76, $P = .0533$) and 2.38 1.02 –5.54, $P = .0444$) for Bgl II (+/−) and Bgl II (+/+) genotypes, respectively (see Table 2).

Table 3. Odds ratios adjusted by multiple logistic regression analysis for the association with retinopathy among patients with type II diabetes mellitus

Independent Variables	Odds Ratio (95% CI)	*P* Value
Bgl II genotype*	1.515 (1.020-2.251)	.0397
Sex*	0.865 (0.498-1.503)	.6069
Age at diagnosis of diabetes†	1.010 (0.979-1.042)	.5360
Diabetes duration after diagnosis†	1.021 (0.982-1.061)	.2929
BMI at diagnosis of diabetes†	1.016 (0.944-1.094)	.6734

Dependent variable was the presence or absence of retinopathy.
*Categorical variable.
†Quantitative variable.
BMI indicates body mass index.

Increased frequency of Bgl II (+)-containing allele was observed in all stages of diabetic microangiopathies, that is, genotype distributions did not differ significantly among different stages of retinopathy and nephropathy (data not shown).

As shown in Table 3, a multiple logistic regression model with a dependent variable (presence or absence of retinopathy) and several independent variables (Bgl II genotype, sex, age at diagnosis of diabetes, disease duration after diagnosis of diabetes, and BMI at diagnosis of diabetes) had an adjusted OR of 1.515 (95% CI, 1.020-2.251, $P = .0397$) for the relation between retinopathy and the presence of the Bgl (+) allele, suggesting that the Bgl II genotype is an independent predictor of retinopathy. To examine whether the relation of the Bgl II genotype to nephropathy shown in Table 2 depended on the relationship between the genotype and retinopathy, we analyzed the genotype distribution only in patients with retinopathy, comparing those with and without nephropathy. The genotype frequency for Bgl II (+/+, +/−) was 71.4% and 65.7% for group C and group B, respectively, which were not significantly different ($P > .05$). Thus, the Bgl II genotype is associated with the prevalence of retinopathy independent of the other variables, but it is not independently associated with the prevalence of nephropathy.

Current disease status of patients in relation to the Bgl II genotype is shown in Table 4. No significant difference was observed among 3 Bgl II genotypes with regard to HbA_{1C}, prevalence of antihypertensive drug treatment, or hyperlipidemia, either in group A patients or group B plus C patients although the mean of HbA_{1C} and the prevalence of antihypertensive drug treatment in group B plus C patients were higher than those in group A patients.

We next analyzed the genotype distribution of the PLA1/A2 polymorphism among 154 diabetic patients (82 with retinopathy, 72 without retinopathy). However, we found no subject with the PLA1/A2 or PLA2/A2 genotypes, that is, all patients had the PLA1/A1 genotype. This result is consistent with previous reports that the PLA2 allele is rare in Japan.[28,29]

Discussion

Several epidemiologic and experimental studies indicate that disease duration of diabetes and glycemic control are major determinants for the development of diabetic retinopathy or nephropathy.[30-32] In the presence of prolonged hyperglycemia, alteration in retinal or renal blood flow, metabolic changes, hemostatic abnormality, or nonenzymatic glycosylation of long-lived tissue proteins are observed.[14,33-36] These changes are associated with vascular dysfunction in microcirculation,[13,36-40] which are thought to contribute to the occurrence or progression of diabetic retinopathy and nephropathy.

Platelets might be involved in the occurrence of diabetic retinopathy and nephropathy. Hyperreactive platelets in diabetic patients would be more likely to interact with an exposed subendothelium of damaged vessels and enhance microthrombus formation or small vessel occlusion in vivo. This, in turn, might alter retinal or renal blood flow. Moreover, evidence indicating the beneficial effect of antiplatelet therapy[18-21] on retinopathy and nephropathy suggests the involvement of platelets in the pathogenesis of microangiopathy. The focus in the present study was on platelet adhesion, which is a first critical step for primary thrombus formation and leads to intracellular activation processes.

This study shows that the Bgl II polymorphism is associated with the prevalence of retinopathy among patients with type II diabetes mellitus. The Bgl II (+/+, +/−) genotypes increased the risk of retinopathy and nephropathy, but the association between the Bgl II genotype and nephropathy was not independent of retinopathy. However, because the majority of diabetic patients with retinopathy suffer from concomitant nephropathy, the Bgl II genotype could be also regarded as a predictor of nephropathy. The Bgl II polymorphism has been reportedly associated with platelet α2β1 density, the extent of platelet adhesion to collagen, and the prevalence of myocardial infarction or stroke.[7,8,10-12] This study is the first to demonstrate an association between the Bgl II polymorphism and diabetic retinopathy.

The amount of nonenzymatically glycosylated collagen, which more easily interacted with platelets, was higher in diabetic patients than in nondiabetic controls.[33,41] It is possible that Bgl II (+)-containing platelets can more easily interact with nonenzymatically glycosylated collagen and accelerate the occurrence of retinopathy. In addition, our findings might affect antiplatelet drug treatment for diabetic retinopathy. For instance, patients with the Bgl II (+/−, +/+) genotype might benefit more from antiplatelet therapies. Although the role for α2β1 integrin in the development of diabetic retinopathy is not fully understood, our data suggest a contribution of platelets in the development of diabetic retinopathy.

Table 4. Current disease status of patients with type II diabetes mellitus

	Bgl II (+/+)	Bgl II (+/−)	Bgl II (−/−)	*P* Value
Patients without retinopathy or nephropathy (group A)				
HbA_{1C} (%, mean ± SD)	7.1 ± 1.2	7.8 ± 1.4	7.6 ± 1.3	.2620*
Prevalence of antihypertensive drug treatment (%)	38.5	23.4	14.6	.1572†
Hyperlipidemia (%)	46.2	51.1	47.9	.9303†
Patients with retinopathy or nephropathy (group B + C)				
HbA_{1C} (%, mean ± SD)	8.3 ± 1.6	7.9 ± 1.5	8.3 ± 1.6	.3827*
Prevalence of antihypertensive drug treatment (%)	58.3	50.8	63.9	.4502†
Hyperlipidemia (%)	25.0	42.4	47.2	.2063†

*Calculated by ANOVA, differences in values are compared among the three genotypes in patients without retinopathy or nephropathy, or among the three genotypes in patients with retinopathy or nephropathy.

†Calculated by chi-square test, 2 × 3 contingency table.

The PLA1/A2 polymorphism has recently been highlighted because of its association with thrombotic disorders.[42] The PLA2 allele, however, was reported to be extremely rare in Japanese populations.[28,29] In agreement with these reports, we did not observe the genotypes with the PLA2 allele among 154 diabetic patients. Therefore, it remains unclear whether the PLA1/A2 polymorphism might affect the susceptibility to microangiopathy in patients with type II diabetes mellitus.

In conclusion, Bgl II polymorphism of the α subunit of α2β1 integrin is associated with the prevalence of retinopathy in patients with type II diabetes mellitus. The present study will aid in early identification of risk or possibly prevention and will direct studies on more beneficial uses of antiplatelet drugs for diabetic microangiopathy.

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References

1. Santoro SA, Zutter MM. The α2β1 integrin: a collagen receptor on platelets and other cells. Thromb Haemost. 1995;74:813.
2. Sixma JJ, Zanten GH, Huizinga EG, et al. Platelet adhesion to collagen: an update. Thromb Haemost. 1997;78:434.
3. Moroi M, Jung SM. Platelet receptor for collagen. Thromb Haemost. 1997;78:439.
4. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell. 1992;69:11.
5. Kamata T, Puzon W, Takada Y. Identification of putative ligand binding sites within I domain of integrin α2β1 (VLA-2, CD49b/CD29). J Biol Chem. 1994;269:9659.
6. Takada Y, Hemler ME. The primary structure of the VLA-2/collagen receptor α2 subunit (platelet GPIa): homology to other integrins and the presence of a possible collagen-binding domain. J Cell Biol.1989;109:397.
7. Kritzik M, Savage B, Nugent DJ, Santoso S, Ruggeri ZM, Kunicki TJ. Nucleotide polymorphism in the a2 gene define multiple alleles that are associated with differences in platelet α2β1 density. Blood. 1998;92;2382.
8. Kunicki TJ, Kritzik M, Annis DS, Nugent DJ. Heredity variation in platelet integrin α2β1 density is associated with two silent polymorphisms in the α2 gene coding sequence. Blood. 1997;89:1939.
9. Reiner AP, Aramaki KM, Teramura G, Gaur L. Analysis of platelet glycoprotein Ia (alpha2 integrin) allele frequencies in three North American populations reveals genetic association between nucleotide 807C/T and amino acid 505 Glu/Lys (HPA-5) dimorphisms. Thromb Haemost. 1998;80:449.
10. Carlsson LE, Santoso S, Spitzer C, Kessler C, Greinacher A. The alpha2 gene coding sequence T807/A873 of the platelet collagen receptor integrin alpha2beta1 might be a risk factor for the development of stroke in younger patients. Blood. 1999;93:3583.
11. Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807T gene polymorphism with nonfatal myocardial infarction in younger patients. Blood 1999;93:2449.
12. Moshfegh K, Wuillemin WA, Redondo M, et al. Association of two silent polymorphisms of platelet glycoprotein Ia/IIa receptor with risk of myocardial infarction: a case-control study. Lancet 1999;353:351.
13. Barnett AH. Pathogenesis of diabetic microangiopathy: an overview. Am J Med. 1991; 90:67S.
14. Winocour PD. Platelet abnormalities in diabetes mellitus. Diabetes. 1992;41:26.
15. Mustard JF, Packham MA. Platelets and diabetes mellitus. N Engl J Med. 1984;311:665.

16. Rasi V, Ikkala E, Hekali R, Myllyla G. Factors affecting plasma β-thromboglobulin in diabetes mellitus. Med Biol. 1980;58:269.
17. Dallinger KJC, Jennings PE, Toop MJ, Clyde OHB, Barnet AH. Platelet aggregation and coagulation factors in insulin dependent diabetes with and without microangiopathy. Diabet Med. 1987; 4:44.
18. Powell EDU, Field RA. Diabetic retinopathy and rheumatoid arthritis. Lancet. 1964;2:17.
19. Giustina A, Perini P, Desenzani P, et al. Long-term treatment with the dual antithromboxane agent Picotamide decreases microalbuminuria in normotensive type II diabetic patients. Diabetes. 1998;47:423.
20. Barnett AH, Wakelin K, Leatherdale BA, et al. Specific thromboxane synthetase inhibition and albumin excretion rate in insulin-dependent diabetes. Lancet. 1984;16:1322.
21. The TIMAD Study Group. Ticlopidine treatment reduces the progression of nonproliferative diabetic retinopathy. Arch Ophthalmol. 1990;108: 1577.
22. Faronato PP, Maioli M, Tonolo G, et al. Clustering of albumin excretion rate abnormalities in Caucasian patients with NIDDM. Diabetologia. 1997;40: 816.
23. Trevisan R, Viberti G. Genetic factors in the development of diabetic nephropathy. J Lab Clin Med. 1995;126:342.
24. Unkelbach K, Kalb R, Santoso S, Kroll H, Mueller-Eckhardt C, Kiefel V. Genomic RFLP typing of human platelet alloantigens Zw(PIA), Ko, Bak and Br (HPA-1, 2, 3, 5). Br J Haematol. 1995;89:169.
25. Alberti KGMM, Zimmet PZ. Definition, diagnosis, and classification of diabetes mellitus and its complications—part 1: diagnosis and classification of diabetes mellitus-provisional report of a WHO consultation. Diabet Med.1998;5:539.
26. Olsen S. Identification of non-diabetic glomerular disease in renal biopsies from diabetics—a dilemma. Nephrol Dial Transplant. 1999;14:1846.
27. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryocytes. Nucleic Acid Res. 1976;3:2303.
28. Hato T, Minamoto Y, Fukuyama T, Fujita S. Polymorphisms of HPA-1 through 6 on platelet membrane glycoprotein receptors are not a genetic risk factor for myocardial infarction in the Japanese population. Am J Cardiol. 1997;80:1222.
29. Tanaka S, Ohnoki S, Shibata H, Okubo Y, Yamaguchi H, Shibata Y. Gene frequencies of human platelet antigens on glycoprotein IIIa in Japanese. Transfusion. 1996;36:813.
30. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977.
31. Cohan AJ, Mcgill PD, Rossetti RG, Guberski DL, Like AA. Glomerulopathy in spontaneously diabetic rat. Diabetes. 1987;36:944.
32. Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. Diabetes. 1987;36:808.
33. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Korn RR. Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. N Engl J Med 1986;314:403.
34. Patel V, Rassam S, Newsom R, Wiek J, Kohner E. Retinal blood flow in diabetic retinopathy. Brit Med J. 1992;305:678.
35. Brown DM, Steffes MW, Thibert P, Azar S, Mauer SM. Glomerular manifestations of diabetes in the BB rat. Matabolism. 1983;32:131.
36. Koya D, Jirousek MR, Lin Y, Ishii H, Kuboki K, King GL. Characterization of proteinkinase Cβ isoform activation on the gene expression of transforming growth factor-β, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. J Clin Invest. 1997;100:115.
37. Skolnik EY, Yang Z, Makita Z, Radoff S, Kirstein M, Vlassara H. Human and rat mesanginal cell receptors for glucose-modified proteins: potential role in kidney tissue remodelling and diabetic nephropathy. J Exp Med. 1991;174:931.
38. Kohner EM, Patel V, Rassam SMB. Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. Diabetes. 1995;44: 603.
39. Aiello LP, Cavallerano JD, Gardner TW, et al. Diabetic retinopathy. Diabetes Care. 1998;21:143.
40. Remuzzi G, Bertani T. Pathophisiology of progressive nephropathies. N Engl J Med. 1998;339: 1448.
41. Pape AL, Gutman N, Guitton JD, Legrand Y, Muh JP. Non enzymatic glycosylation increases platelet aggregating potency of collagen from placenta of diabetic human beings. Biochem Biophys Res Commun. 1983;111:602.
42. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med. 1996;334:1090.