

Contribution of the -455G/A Polymorphism at the β -fibrinogen Gene to Erythrocyte Aggregation in Patients with Coronary Artery Disease

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Key words

Erythrocyte aggregation, β -fibrinogen gene, genetics, coronary artery disease, fibrinogen, lipids, lipoproteins

Summary

Background. A high level of red blood cell (RBC) aggregation has been consistently found in patients with coronary artery disease (CAD) in case-control studies. Plasma fibrinogen has been shown to promote RBC aggregability. The purpose of this study was to investigate the influence of the genetic variability of the β -fibrinogen gene on RBC aggregation in patients with CAD. **Methods and Results.** The genotype of the β -fibrinogen gene locus was determined by polymerase chain reaction using the restriction enzyme *Hae*III for a G to A substitution at position -455 upstream from the transcriptional start site in 135 French Canadians with premature CAD (age: 51 ± 7 years). Indices measuring the RBC aggregation kinetics (S_{10}) and shear resistance of the aggregates (γS) were obtained by laser reflectometry. Patients were separated into groups by using the medians of S_{10} and γS . Using χ^2 analyses, the distribution of the -455GG, -455GA, and -455AA genotypes in the groups with high levels of S_{10} (0.43, 0.49, and 0.08) and γS (0.45, 0.49, and 0.06) were found to be significantly distinct from those in the groups with low levels of S_{10} (0.67, 0.27, and 0.06; $p < 0.05$) and γS (0.70, 0.23, and 0.07; $p < 0.01$). High levels of RBC aggregation were closely associated with the rare -455A allele. Multivariate linear regression analyses showed that S_{10} was positively correlated with the linear combination of the fibrinogen concentration, age, and the -455G/A genotype (adjusted $r = 0.63$, $p < 0.0001$). Fibrinogen and age were positive determinants, and HDL-cholesterol was a negative predictor of γS (adjusted $r = 0.51$, $p < 0.0001$). **Conclusion.** These findings support the hypothesis that RBC hyperaggregation in premature CAD may be associated with the β -fibrinogen -455G/A polymorphism. This association may be explained by a change in the concentration and/or the functional properties of the fibrinogen protein.

Introduction

Indices of increased red blood cell (RBC) aggregation have received increasing interest in recent years because of the implication of microvascular rheological disorders and thrombosis in the development of

coronary artery disease (CAD). A growing body of clinical evidence has supported a relationship between RBC hyperaggregation and CAD (1-5). Pathological RBC aggregation characterized by large aggregates with strong intercellular links is involved in microcirculatory sludging and stagnation, which may alter peripheral vascular resistance, reduces capillary perfusion and oxygen transfer to tissues, and causes ischemia, local metabolic acidosis, degeneration of the vascular wall, and tissue infarction (6-13). Although increased RBC aggregation at low shear conditions is believed to have a direct effect on thrombotic venous disorders (14), an indirect influence on the formation of arterial thrombi was also suggested through its interaction with platelets (14, 15). A mechanism by which RBC aggregation can affect thrombosis is by its impact on the displacement of blood cells toward the arterial wall (16). Very recently (17), leukocyte margination was shown to be greatly influenced by RBC aggregation at shear rates below 350 s^{-1} .

The key role of fibrinogen on RBC aggregation is well recognized and it is largely accepted as one of the most important determinant of RBC aggregation (18-20). To date however, the exact mechanisms of interaction between fibrinogen and RBCs are not fully understood. Evidence from *in vitro* experiments and clinical studies showed that the aggregative effect of fibrinogen is amplified at increasing concentrations (20-23). As a macromolecular protein, fibrinogen may form a bridging structure between adjacent erythrocytes that leads to RBC aggregation (19). The depletion of fibrinogen from the intracellular space may be another mechanism.

Plasma fibrinogen isolated from diabetic patients and normal controls was recently shown to provide different effects on RBC aggregation when mixed, at the same concentration, with a saline suspension of normal RBCs from a single donor (24). It was concluded that these differences may be associated with changes in the functional capability of fibrinogen to bind RBCs between normal subjects and diabetic patients. Structural abnormalities in the fibrinogen molecule was also shown to have a major impact on RBC aggregation in a case report study (25). The α -helical content of the fibrinogen protein was increased and the β -sheet content was decreased. In that study, the presence of digital ischemia and gangrene due to RBC aggregation was not related to a family history of dysfibrinogenemia. It can thus be postulated that besides the concentration and mass, the functional capability and structure of fibrinogen can also influence RBC aggregation.

Human plasma fibrinogen is a dimer, with each half of the molecule consisting of three nonidentical polypeptide chains $\text{A}\alpha$, $\text{B}\beta$ and γ (26). The three chains are encoded by different genes, named α (FGA), β (FGB) and γ (FGG), that are clustered on the distal third of the long arm of chromosome 4 within a 45 kb segment of DNA (27). At least ten polymorphic sites in the β -fibrinogen gene were identified (28). Using

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the restriction enzyme *HaeIII*, a common polymorphism (-455G/A) in the promoter region of the β -fibrinogen gene was shown to be associated with plasma fibrinogen levels in patients with CAD (29), patients with myocardial infarction and healthy controls (28, 30), healthy individuals following physical exercise (31), healthy smokers and non-smokers (32), and the general Danish population (33). In these studies, the rare -455A allele was associated with elevated plasma fibrinogen levels. The contribution of genetic variability within the fibrinogen locus to plasma fibrinogen levels varied from 1 to 15% (29, 30, 32, 34). Contradictory results indicating no significant association between plasma fibrinogen levels and the -455G/A polymorphism of the β -fibrinogen gene were also reported in patients with peripheral arterial occlusive disease and healthy controls (35), patients with CAD (36, 37), and patients with non-insulin-dependent diabetes mellitus and CAD (38). Other major determinants of the plasma fibrinogen concentration are cigarette smoking, age, gender, body mass index, serum total cholesterol, plasma lipoproteins, ethnicity, diabetes, and inflammation (20, 39-41). Fibrinogen is an acute-phase reactant and an independent predictor of CAD (42, 43).

The purpose of this study was to address the association between the genetic variability of the β -fibrinogen gene locus and erythrocyte aggregation levels. It was hypothesized that the -455G/A polymorphism of the β -fibrinogen gene may be associated with RBC aggregation by altering the plasma fibrinogen concentration and/or its functional capability. In addition, the influence of the blood lipid profile including total cholesterol (TC), triglycerides (TG), low (LDL-C) and high (HDL-C) density lipoprotein cholesterol, and clotting factors were also tested to provide an insight into the correlation of these parameters with RBC aggregation.

Methods

Study Population

The study population consisted of 135 white French Canadian of Quebec (25 women) with premature documented CAD. These subjects were identified and recruited from the Cardiology Clinic of the Clinical Research Institute of Montreal. All patients were diagnosed as having CAD by angiography (at least one epicardial coronary artery with a $\geq 50\%$ stenosis) or an acute myocardial infarction before the age of 60 years. The mean age of patients at the time of the study was 51 ± 7 years (mean \pm SD, range of 22 to 64 years); thirty-two percent were active smokers. For each patient, information on age, gender, smoking habits, body mass index (BMI: weight/height² in kg/m²), medical history, plasma lipids, and lipoprotein cholesterol was available, as described in (44).

The ethic committee of our Institution has reviewed and approved the protocol for blood and plasma analyses, as well as DNA isolation and analysis of genes involved in the pathogenesis of CAD. All patients signed separate consent forms for blood sampling and DNA isolation.

Fibrinogen Genotype Analysis

Venous blood samples were drawn by venipuncture at least 3 months after angiography, acute myocardial infarction, or coronary artery bypass surgery. The -455G/A polymorphism of the β -fibrinogen promoter region was identified using a method similar to that of Thomas et al. (32). Briefly, DNA was isolated from white blood cells by Qiagen-100 columns (Qiagen Inc.). A portion of 1301 bp (-1178 to +122) in the 5'-flanking region of the β -fibrinogen gene was amplified by the polymerase chain reaction (PCR) technique using oligonucleotide primers: AAGAATTTGGGAATGCAATCTCTGCTACCT (forward primer) and CTCCTCATTGTCGTTGACACCTTGGGAC (reverse primer). Each PCR reaction of 50 μ l contained 2 μ l (100-400 ng) of genomic DNA, 150 ng of each primer, 200 μ M dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris/HCl (pH 9.0), 0.1% Triton X-100, and 2.5 units of Taq DNA poly-

merase (Sangon Ltd.). The reaction was performed over 30 repeated cycles with a deannealing temperature of 95° C for 1 min and an annealing and prolongation temperature of 72° C for 2 min. At the beginning and the end of these cycles, temperatures of 95° C and 72° C for 5 min were used, respectively. Ten μ l of PCR product was then digested with 10 units of the restriction enzyme *HaeIII* (Pharmacia Biotech) for 2 h at 37° C. The digested products were separated by electrophoresis on 1.5% agarose gel in 40 mM Tris-acetate, 1 mM EDTA, 0.5 μ g/ml ethidium bromide (pH 8.0), and visualized with ultra violet light. The genotype was determined for a G \rightarrow A mutation at position -455 bp upstream from the start of transcription of the β -fibrinogen gene (32, 37). The -455G/A genotype was classified as GG, GA, and AA.

Determination of Erythrocyte Aggregation

A sample of blood containing EDTA as the anticoagulant (1.5 g/l) was used to determine the erythrocyte aggregation levels. All measurements were performed within 4 h after blood collection. The blood samples were centrifuged at 1,300 g for 10 min and the hematocrit was adjusted at 0.40 by the addition or subtraction of autologous plasma. The hematocrit was measured using a microcentrifuge (Haemofuge, Heraeus Instruments) operating at 14,980 g for 10 min. The blood samples were incubated in a warm bath (37° C) for 5 min before measuring the erythrocyte aggregability.

RBC aggregation measurements were performed at 37° C with a previously validated erythroaggregometer based on a Couette flow arrangement (Regulest, Florange, France) (45, 46). The blood sample of 1.5 ml was placed into a chamber between two co-axial cylinders and the outside cylinder was rotated to provide different shear rate conditions (range of 6 to 720 s⁻¹). Two RBC aggregation indices, the aggregation index (S₁₀, no unit) and the total dissociation threshold (γ S in s⁻¹), were determined by analyzing the variations in light intensity scattered by blood as a function of time and shear rate, respectively. S₁₀ gives information on the kinetics of RBC rouleau formation, whereas γ S corresponds to the minimum shear rate at which red blood cells are completely dissociated. This last parameter provides information on the adhesive strength between RBCs. Reported intra-assay variabilities ranged from 2% to 2.3% for S₁₀, and 8% to 12.9% for γ S (47).

Biochemical Analyses

The concentration of fibrinogen (Fib) in citrated plasma was measured with a photo-optical instrument (ACL 3000, Coulter Corporation, Florida). The coefficient of variability of this method is 2.2% within run and 3.1% overall, approximately. The prothrombin time that reflects the activity of coagulation factors II, VII, and X was determined by using the same instrument and the reagent Innovin® (Dade International Inc.). This reagent was prepared from purified recombinant human tissue factor produced in *E. coli* and combined with synthetic phospholipids (thromboplastin), calcium, buffers, and stabilizers. Innovin® initiates clotting via the extrinsic and common pathways in a global screening test, the prothrombin time (PT). The International Normalized Ratio (INR) was determined according to the following equation:

$$\text{INR} = R^{\text{ISI}}, \text{ where } R = \frac{\text{Patient's PT}}{\text{Mean Normal PT}}$$

ISI is the International Sensitivity Index of the reagent/instrument combination.

Enzymatic methods were used to measure the total serum cholesterol (TC) and triglyceride (TG) concentrations. The high-density lipoprotein cholesterol (HDL-C) concentration was determined after precipitation of apo B - containing lipoproteins by dextran sulphate/MgCl₂. The low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald formula for patients with a triglyceride concentration below 4.5 mmol/l.

Statistical Analyses

Patients were separated into different groups by using the medians of S₁₀ and γ S, respectively. Two other groups separated by the median of Fib were

Table 1 Characteristics of various subgroups of patients with CAD (Mean ± SEM). The age, gender, body mass index and smoking habits were used as covariates when assessing the difference of each parameter between the low and high median groups

Variables	All (n=135)	S _{10L} (n=70)	S _{10H} (n=65)	γSL (n=87)	γSH (n=78)	FibL (n=67)	FibH (n=68)
S ₁₀	28.2 ± 0.3	25.3 ± 0.4***	31.1 ± 0.3	25.2 ± 0.4***	30.2 ± 0.3	26.4 ± 0.5***	29.2 ± 0.4
γS (second ⁻¹)	153.1 ± 3.5	134.4 ± 3.8***	174.4 ± 4.8	119.4 ± 2.1***	178.3 ± 3.9	140.2 ± 4.0***	165.4 ± 5.4
Fib (g/l)	3.5 ± 0.1	3.0 ± 0.1***	3.9 ± 0.1	3.1 ± 0.1**	3.7 ± 0.1	2.8 ± 0.1***	4.2 ± 0.1
INR	1.10 ± 0.03	1.01 ± 0.02	1.1 ± 0.1	1.02 ± 0.03	1.1 ± 0.1	1.02 ± 0.01	1.1 ± 0.1
TC (mmol/l)	5.4 ± 0.1	5.3 ± 0.1	5.5 ± 0.2	5.3 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	5.2 ± 0.1
TG (mmol/l)	2.4 ± 0.1	2.2 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	2.0 ± 0.1	2.3 ± 0.1	2.5 ± 0.2
HDL-C (mmol/l)	1.01 ± 0.03	1.10 ± 0.04	1.01 ± 0.04	1.13 ± 0.04	1.01 ± 0.03	1.03 ± 0.02*	1.13 ± 0.04
LDL-C (mmol/l)	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.0 ± 0.1

***p < 0.001; **p < 0.01; *p < 0.05

Table 2 β-fibrinogen -455G/A genotype and A allele frequency between the various subgroups of patients with CAD

Genotypes	S _{10L} (n=70)	S _{10H} (n=65)	γSL (n=57)	γSH (n=78)	FibL (n=67)	FibH (n=68)
-455GG	0.67*	0.43	0.70**	0.45	0.63	0.49
-455GA	0.27*	0.49	0.23**	0.49	0.31	0.44
-455AA	0.06*	0.08	0.07**	0.06	0.06	0.07
-455A allele	0.19**	0.32	0.18*	0.31	0.22	0.29

**p < 0.01; *p < 0.05

also created to test the influence of the -455G/A polymorphism of the β-fibrinogen gene on the Fib concentration. The General Linear Model (GLM, analysis of variance) using age, gender, BMI and smoking habits as covariates was used to assess differences in the clotting factor INR, lipids TC and TG, and lipoproteins HDL-C and LDL-C between the low and high median groups (Table 1). The Chi-square (χ²) test was used to compare the -455G/A genotype and A allele frequency between the low and high median groups (Table 2). The General Linear Model using age, gender, BMI and smoking habits as covariates was selected to assess differences in S₁₀, γS, and Fib levels between homozygous individuals with the -455G allele (genotype GG), and those carrying at least one -455A allele (genotypes -455GA and -455AA) (Fig. 1). Stepwise multiple linear regression analyses were used to estimate the contribution, to erythrocyte aggregation (S₁₀, γS) and plasma fibrinogen concentration, of the -455G/A genotype, biochemical, and physiological variables measured in this study. For statistical inference, a value of p < 0.05 was considered significant. Data were expressed as mean ± standard error of the mean (SEM). For the GLM and linear regressions, all results passed the normality and constant variance tests. Statistical analyses were performed using the Minitab statistical software (version 10.2).

Results

Comparison of Variables between Groups

RBC aggregation parameters and biochemical variables are shown in Table 1. Patients were separated in low (L) and high (H) groups by using the medians of S₁₀ (28), γS (145 s⁻¹), and Fib (3.3 g/l), respectively. The statistical significance of the test was given for S₁₀, γS and Fib although differences were obviously expected between groups. As seen

in Table 1, patients in the H groups of RBC aggregation (S_{10H} and γSH) had statistically significantly higher levels of plasma fibrinogen compared to patients in the low S₁₀ (S_{10L}) and low γS (γSL) groups. Significantly higher levels of S₁₀, γS, and HDL-C were found in the high Fib group (FibH) when compared to the low Fib group (FibL). These significant variations were independent of age, gender, smoking habits, and BMI. Logarithmic transformed data were used when necessary.

Distribution of the β-fibrinogen -455G/A Genotype and A Allele Frequency in Patients with Low and High Levels of S₁₀, γS and Fib

As shown in Table 2, the genotype distribution in the S_{10H} group was significantly distinct from that in the S_{10L} group (p < 0.05). The frequency of the A allele was also significantly different (p = 0.01) between the S_{10H} and S_{10L} groups. Regarding groups defined with the median of γS, the genotype distribution was significantly different (p < 0.01) from that found in the γSL group. The -455A allele frequency was statistically different between both groups (p < 0.05). No significant difference was observed in the distributions of the -455G/A genotype and -455A allele between FibH and FibL groups.

Effects of the -455G/A Genotype on Erythrocyte Aggregation and Plasma Fibrinogen Concentration

Fig. 1 shows the association of -455GG and -455GA/AA genotypes with RBC aggregation levels (S₁₀ and γS), and plasma fibrinogen concentrations following adjustment for differences in age, gender, smoking habits, and BMI. Significantly higher levels of S₁₀ (28.6 ± 0.5, p < 0.05) and γS (162 ± 5 s⁻¹, p < 0.05) were observed in patients carrying -455GA/AA genotypes when compared to the values of 26.8 ± 0.4 for S₁₀ and 146 ± 5 s⁻¹ for γS in patients with the -455GG genotype. A non significant increase in the plasma fibrinogen concentration was found in patients with -455GA/AA genotypes (3.6 ± 0.1, p = 0.33) when compared to the values of 3.4 ± 0.1 for patients with the -455GG genotype.

Correlates of the -455G/A Genotype, Biochemical and Physiological Variables with Erythrocyte Aggregation and Plasma Fibrinogen Concentration

Multivariate stepwise linear regression models including the -455G/A genotype, the clotting factors Fib and INR, TC, TG, HDL-C, LDL-C, age, gender, BMI, and smoking habits as independent variables were tested to assess their relationship with RBC aggregation (S₁₀ and γS) in all patients. The linear combination of Fib (p < 0.0001), age (p < 0.005), as well as the -455G/A genotype (p < 0.05) was significantly correlated with S₁₀ (S₁₀ = 2.00 Fib + 0.16 age + 0.94 genotype + 11.24, adjusted r = 0.63, p < 0.0001, SEE = 3.11). For γS, Fib (p < 0.0001) and age (p < 0.05) were significant positive predictors, whereas HDL-C was

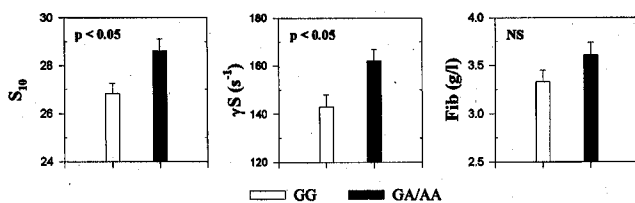


Fig. 1 Influence of the -455G/A genotype of the β-fibrinogen gene on RBC aggregation (S₁₀ and γS), and plasma fibrinogen concentration (Fib). The age, gender, body mass index and smoking habits were used as covariates when assessing the difference of each parameter between the -455GG and -455GA/AA genotypes (Mean ± SEM).

a significant negative determinant ($p < 0.05$) ($\gamma S = 18.2 \text{ Fib} + 1.0 \text{ age} - 25.1 \text{ HDL-C} + 65.6$, adjusted $r = 0.51$, $p < 0.0001$, $SEE = 35 \text{ s}^{-1}$). For Fib, among all independent variables (-455G/A genotype, INR, TC, TG, HDL-C, LDL-C, age, gender, BMI, and smoking habits), BMI ($p < 0.01$) and smoking ($p < 0.05$) turned out to be significant determinants of the plasma fibrinogen level ($\text{Fib} = 0.04 \text{ BMI} + 0.37 \text{ smoking} + 2.1$, adjusted $r = 0.24$, $p < 0.01$, $SEE = 1.00 \text{ g/l}$).

Discussion

The present study focused on the genetics of erythrocyte aggregation and demonstrated an association between the -455G/A polymorphism of the β -fibrinogen gene and RBC hyperaggregation in patients with coronary artery disease. The distributions of the -455G/A polymorphism of the β -fibrinogen gene were in Hardy-Weinberg equilibrium in our database. The relative frequency of the rare -455A allele in patients with premature CAD was 25%, which is consistent with a previously reported frequency of 25% in both young survivors of myocardial infarction and healthy controls from Sweden (37). Other studies reported a -455A allele frequency of 19% in healthy men from UK (32, 48), 22% in cases of offspring of fathers with myocardial infarction before the age of 55 years and healthy controls from five European regions (40), and 22% in patients with CAD from Germany (29). Results obtained by another European group showed a frequency of 21% in controls and 19% in patients with myocardial infarction (28, 30).

In order to investigate whether there was an effect of the β -fibrinogen -455G/A polymorphism on the erythrocyte aggregation levels and the plasma fibrinogen concentration, patients were separated into groups using the medians of S_{10} , γS and Fib, respectively. Statistically significant differences in the distributions of the -455G/A polymorphism were found between groups with high and low levels of RBC aggregation (both S_{10} and γS). Both the kinetics of aggregation and the adhesive strength between RBCs were found to be associated with the rare -455A allele. On the other hand, the influence of this allele on the plasma fibrinogen concentration was not significant.

Independent Variables Affecting S_{10} , γS and Fib (Multiple Stepwise Linear Regression Models)

The fibrinogen concentration and age were included in the multiple linear regression models of S_{10} and γS . The influence of Fib on RBC aggregation was expected. In normal subjects, S_{10} and γS have also been reported to be positively correlated with age (49). However in that study, the differences in the means computed for four groups of age were only significant for subjects older than 50 years. In (50), RBC aggregation in healthy subjects was similar in men younger and older than 50 years. On the other hand, in women, menopause had a significant effect on RBC aggregation. To our knowledge, no study reported an independent aging effect on RBC aggregation in patients with CAD.

The -455G/A polymorphism contributed to the model predicting the kinetics of RBC aggregation. No study showing a genetic contribution to RBC aggregation has ever been reported. Besides fibrinogen and age, the adhesive strength between RBCs was related to HDL-C. The negative independent association between γS and HDL-C is in agreement with recent results obtained in hyperlipidemia by our group (51), hypercholesterolemia (23), and CAD (1). Our results support previous findings (29, 32) on an association between the plasma fibrinogen concentration, BMI, and cigarette smoking.

Influence of the -455G/A Polymorphism on the Plasma Fibrinogen Concentration

No significant effect of the -455G/A polymorphism of the β -fibrinogen gene on the plasma fibrinogen level was observed, which is in contradiction with observations performed in CAD patients by Heinrich et al. ($n = 384$ patients from Germany) (29), Scarabin et al. ($n = 533$ patients from Ireland and France) (30), and Behague et al. ($n = 565$ patients from Ireland and France) (28). However, our results on 135 white French Canadians with premature CAD are consistent with other studies on young survivors of myocardial infarction ($n = 123$ patients from Sweden) (37), non-insulin-dependent diabetes mellitus patients with CAD ($n = 187$ Caucasian patients) (38), and white CAD patients from Australia ($n = 545$) (36). In that last study, the plasma fibrinogen level was positively associated with environmental factors such as the lifetime smoking dose and not by the -455G/A genotype of the β -fibrinogen gene. In our study, smoking was also independently associated with the Fib concentration, which may suggest an interaction between the -455G/A polymorphism and smoking in determining the plasma fibrinogen concentration (37).

Possible Explanation for the Influence of the -455G/A Polymorphism on RBC Aggregation

The following possible molecular mechanisms are proposed to explain the effect of the genetic variability of the β -fibrinogen gene on the fibrinogen molecule and then on erythrocyte aggregation. As reported (29, 48, 52), the -455G/A polymorphism is in strong linkage disequilibrium with the -148C/T (*HindIII*), the +1689T/G (*AvaII*), the *BclI*, the *AluI*, and the *MnII* polymorphisms of the β -fibrinogen gene. Therefore, the -455G/A polymorphism as a neutral marker is able to provide more information on potential changes of the β -fibrinogen DNA sequence. It is thus hypothesized that combinative variations of the β -fibrinogen gene may change the functional properties of the protein. For instance, linkage relationship between the β -fibrinogen -455G/A polymorphism and the coding region polymorphism of *MnII* RFLP within the β -fibrinogen codon 488 (AGG/AAG, Arg/Lys) could support this hypothesis (52). In addition, the *BclI* polymorphism of the β -fibrinogen was shown to be associated with the severity of CAD (28). This association appeared to be independent of the influence of the polymorphism on plasma fibrinogen levels, which may also implicate some effects of the *BclI* polymorphism on functional changes of plasma fibrinogen.

Human plasma fibrinogen is an acute-phase protein synthesized by hepatocytes. The β -fibrinogen transcription is regulated positively by cytokines, especially interleukin-6 (IL-6) in response to inflammation as well as a wide variety of clinical conditions (39, 53). During the acute-phase of inflammation, an increase of IL-6 can stimulate the synthesis of Fib through binding of positive transcriptional factors to the IL-6 responsive element in the β -fibrinogen gene (53, 54). The *HindIII* polymorphism caused by a C to T substitution at -148 bp from the transcriptional start of the β -fibrinogen gene was identified as being located within the IL-6 responsive region (32, 53). Therefore, a possible mechanism is that the -148C/T and/or the -455G/A polymorphism of the β -fibrinogen gene promoter alter the affinity of the gene to nuclear proteins (55), which would contribute to the higher level of plasma Fib observed in patients with the -455A allele when compared to that of the -455GG genotype (although not significant in our study).

Conclusion

The findings of this study support the hypothesis that RBC aggregation in patients with CAD is influenced by the genetic variability of the β -fibrinogen gene. Because the level of aggregation could not fully be explained by the concentration of fibrinogen, genetic factors seem to contribute to the RBC aggregation variability between individuals. To evaluate the effects of the genetic components and plasma fibrinogen concentration on RBC aggregation, patients were separated into groups based on the medians of S_{10} and γS , respectively. The distributions of the -455G/A genotype and frequency of the A allele in the high S_{10} and γS groups were significantly distinct from those in the low S_{10} and γS groups. High levels of RBC aggregation (both S_{10} and γS) were associated with the -455A allele. The stepwise multiple linear regression analyses including the -455G/A genotype, clotting factors, lipid profile, and physiological variables confirmed that the -455G/A genotype is independently associated with the RBC aggregation kinetics. The adhesive strength between RBCs (γS) was positively correlated with the linear combination of Fib and age, and negatively associated with HDL-C. The regression models failed to detect a significant association between the genetic variability and plasma fibrinogen levels. This study suggests that the ability of fibrinogen to bind RBCs may be influenced by the -455G/A polymorphism of the β -fibrinogen gene.

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