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Genetic, clinical and experimental aspects of restenosis : a biomedical perspective

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-455 G/A POLYMORPHISM AND PREPROCEDURAL PLASMA LEVELS OF FIBRINOGEN SHOW NO ASSOCIATION WITH THE RISK OF CLINICAL RESTENOSIS IN PATIENTS WITH CORONARY STENT PLACEMENT

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Abstract

Background

The effect of preprocedural fibrinogen levels on in-stent restenosis is largely unknown. The -455G/A polymorphism of the fibrinogen β -gene is associated with baseline plasma level or acute phase increase of fibrinogen. Therefore, we hypothesized that there is a relationship between this polymorphism and preprocedural fibrinogen level and clinical restenosis at follow-up among patients with coronary stent placement.

Methods

The GENetic DEterminants of Restenosis (GENDER) project is a multicenter follow-up study that enrolled 3,146 consecutive patients after successful percutaneous coronary intervention. A coronary stent was placed in 2,309 patients. Of these, 2,257 (97.7%) were successfully genotyped for the -455G/A polymorphism. Plasma fibrinogen levels were measured at baseline in a subpopulation of 623 stented patients with the von Clauss method and patients were grouped into tertiles according to fibrinogen levels.

Primary endpoint was target vessel revascularization (TVR); secondary combined endpoint was defined as death presumably from cardiac causes, MI not attributable to another coronary artery than the target vessel, and TVR.

Results

No association was observed between the -455G/A polymorphism and TVR or combined endpoint ($p=0.99$, $p=0.97$, respectively). Multivariate regression analysis revealed that the risk of TVR and combined endpoint was not higher for patients in the highest tertile for fibrinogen versus the lowest tertile ($RR=0.60$, 95% CI: 0.26-1.37 for TVR, $RR=0.64$, 95% CI: 0.29-1.44 for combined endpoint).

Conclusions

The presence of -455G/A polymorphism in the fibrinogen β -gene and preprocedural fibrinogen level is not associated with an increased risk of TVR or combined endpoint in a patient population with coronary stent placement. Therefore, these parameters are not worthwhile for stratifying patients at risk for restenosis pre-stenting.

Introduction

In recent years stent placement during percutaneous coronary intervention (PCI) has been widely adopted for the treatment of coronary artery disease (CAD). Development of restenosis during the year after coronary stent placement remains a significant clinical problem. In order to stratify patients at risk and to optimize tailored therapy for the individual patient, research of mechanisms and risk factors of restenosis is warranted.⁽¹⁾ Restenosis is a multifactor process where recoil of the vessel, neointimal proliferation and thrombus formation play a role.⁽²⁾ Fibrinogen, an acute phase protein, is an important factor of platelet aggregation, causes the release of vasoconstrictor mediators and growth factors, increases plasma viscosity, and contributes to fibrin deposits.⁽³⁾ Moreover, fibrin(ogen) degradation products stimulate smooth muscle cell proliferation, which is seen in neointima formation.⁽⁴⁾ So fibrinogen is involved in both coagulation and inflammation, important processes in restenosis. Several studies have shown a significant association between plasma fibrinogen level and subsequent CAD.⁽⁵⁻⁷⁾ Furthermore, elevated fibrinogen levels after coronary balloon angioplasty have been reported as a risk factor for the development of restenosis.^(4,8) However, there are little and conflicting clinical data available concerning the relationship between baseline fibrinogen levels and coronary events after coronary stenting.⁽⁹⁻¹¹⁾

In addition, genetic polymorphisms might provide more insights in the restenotic process and contribute to the stratification of patients at risk for restenosis. The synthesis of the β -fibrinogen chain in hepatocytes is the rate-limiting step in the overall synthesis of the mature fibrinogen protein.⁽¹²⁾ Genetic variation of this β -fibrinogen gene can contribute to the regulation of plasma fibrinogen levels. The A-allele of the -455G/A promoter polymorphism of the fibrinogen β -gene has been associated with higher levels of plasma fibrinogen.⁽¹³⁾ Whether possession of the A-allele is associated with an increased risk of restenosis after coronary stenting however has not yet been studied.

Therefore, the aim of this study was to examine whether the -455 G/A polymorphism and/or preprocedural levels of fibrinogen have any impact on target vessel revascularization (TVR) or combined endpoint after coronary stent placement. We genotyped patients from the GENetic DEterminants of Restenosis (GENDER)-study, a multicenter follow-up study for the -455G/A polymorphism and studied the preprocedural level of fibrinogen in a subgroup of patients.

Methods

Study design

The GENetic DEterminants of Restenosis study (GENDER), a multicenter follow-up study on 3,146 consecutive patients undergoing PCI, representing a clinical practice population, was designed to evaluate the association between gene polymorphisms and clinical restenosis. The study design has been described previously.⁽¹⁴⁾ In brief, patients were eligible for inclusion if they were successfully treated with PCI for stable angina, non-ST elevation acute coronary syndromes or silent ischemia. Patients treated for acute ST elevation myocardial infarction (MI) were excluded. All patients were treated in four referral centers for interventional cardiology in the Netherlands (Academic Medical Center Amsterdam, Academic Hospital Groningen, Leiden University Medical Center and Academic Hospital Maastricht). The overall inclusion period lasted from March 1999 until June 2001. The study protocol conforms to the Declaration of Helsinki and was approved by the medical ethics committees of each participating institution. Written informed consent was obtained from each participant before the PCI procedure.

Definitions

A PCI procedure was considered successful if on visual inspection the luminal stenosis of at least one lesion was reduced to less than 50% of the luminal diameter. Hypertension was defined as a blood pressure of either above 160 mmHg systolic or 90 mmHg diastolic. Current smokers were individuals who smoked within the month preceding the index intervention. Past smokers were those individuals who gave up smoking in the preceding year. Individuals who stopped smoking for more than one year were classified as non-smokers. Patients using anti-diabetic medication or insulin at study entry were considered to be diabetics. The preprocedural lesions were classified according to the modified American College of Cardiology and American Heart Association Task Force classification.⁽¹⁵⁾

Stenting procedure

Intracoronary stenting was performed with standard techniques using the radial or femoral approach. Before the procedure patients received 300 mg of aspirin and 7,500 IU of heparin. The use of intracoronary stents and additional medication, such as glycoprotein IIb/IIIa inhibitors, was at the discretion of the operator. After placement of the stent patients received either ticlopidin or clopidigrel for at least one month following the procedure, depending on local practice.

Genetic methodology

Blood was collected in tubes containing EDTA at baseline and genomic DNA was extracted following standard procedures. The -455G/A polymorphism was determined by a validated multilocus genotyping assay to test several markers of cardiovascular disease (Roche Molecular Systems). Genotyping was possible in 2,257 (97.7%) of the 2,309 stented patients of the GENDER-population.^(16;17) As quality control, the genotyping procedure was replicated on 10% of the samples, and the results were confirmed. Two independent observers scored the genotypes. Disagreements were resolved by a further joint reading.

Blood samples and laboratory analysis

To study the effect of fibrinogen as a risk factor for restenosis, preprocedural fibrinogen plasma levels were determined in a subpopulation of the GENDER study, consisting of 623 patients who received a stent treated in the Leiden University Medical Center.

Blood samples were drawn prior to each procedure, plasma samples were kept frozen at -80 °C until analysis. Plasma fibrinogen was measured with a modified method according to von Clauss.⁽¹⁸⁾ The laboratory personnel were blinded for the clinical outcome.

Follow-up and study endpoints

Patients were followed for at least nine months. They were either seen in the outpatient clinic of the center for interventional cardiology or contacted by telephone. Primary endpoint was the incidence of target vessel revascularisation (TVR) either by repeat PCI or CABG, which we considered as clinical restenosis. The secondary combined endpoint was defined as death presumably from cardiac causes, MI not attributable to another coronary artery than the target vessel, and TVR. An independent clinical events committee of experienced cardiologists adjudicated the clinical events. The committee members did not review patients treated in their own center. The clinical outcome investigators committee was blinded for the laboratory results.

Events occurring within one month after the PCI were classified and analysed separately, since these events are likely attributable to sub-acute stent thrombosis or occluding dissections rather than restenosis. Data were collected in standardized case-report forms that were completed by the research coordinator.

Statistical methods

We divided the population into tertiles of preprocedural fibrinogen levels with < 3.1 g/L concentration as lowest tertile ($n=196$), with concentrations between 3.1 and 4.1 g/L as second tertile ($n=225$), and a concentration > 4.1 g/L as highest tertile ($n=202$). All data are expressed as mean $\pm$ standard deviation, unless stated otherwise. Event rates were calculated by Kaplan-Meier survival analysis. Time to first clinical event was compared between (sub) groups of patients by the log-rank test. Prognostic values of clinical and procedural variables were assessed by Cox' proportional hazards model. We used Cox regression models to examine the association of fibrinogen levels (lowest tertile vs. others) with risk of TVR and combined endpoint after adjustment for potentially confounding factors. The covariates included in the baseline multivariable model were; age, body mass index (BMI), diabetes, hypertension, stent length, erythrocyte sedimentation rate (ESR) and smoking. The total length of the stented segment and the minimal diameter of the stents were calculated per patient. Deviations of the genotype distribution from that expected for a population in Hardy-Weinberg equilibrium was tested using the Chi-squared test with one degree of freedom. Allele frequency was determined by gene counting, the 95% confidence intervals of the allele frequency was calculated from sample allele frequency, based on the approximation of the binomial and normal distributions in large sample sizes.

In the first stage, the association between the fibrinogen-polymorphism and TVR was assessed using a Cox proportional regression model under a co-dominant genetic model. No adjustment for covariates was performed at this stage to allow for the assessment of the possible involvement in the causal pathway. The polymorphism was also assessed using a dominant and recessive model and the model with the lowest Akaike information criterion was used in multivariable regression analysis.⁽¹⁹⁾

Multivariable regression analysis of the TVR risk was performed on the polymorphism and their potentially confounding factors using a stepwise backward selection algorithm. Analyses were performed with SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL, USA).

A two-sided value of $p < 0.05$ was considered statistical significant.

Results

Baseline patient characteristics

Baseline characteristics of the total stented population, consisting of 2,309 patients and the subpopulation for which plasma fibrinogen levels were available (n=623) are listed in Table 1.

We were able to determine in 2,257 (97.7%) patients of the total stented population the genotypes of the -455G/A polymorphism. Results of the remaining patients (n=52, 2.2%) are missing, due to lack of DNA or inconclusive genotyping. The frequency of the rare -455A allele was 19.8%. The genotype distribution was consistent with the Hardy-Weinberg equilibrium ($p>0.05$).

The subpopulation for which plasma fibrinogen levels were available consists of 745 patients, who were treated in the Leiden University Medical Center. Of these patients, 623 (83.6%) received a stent. The mean age of the patients was 61.9 ± 10.7 years and they were followed for 9.1 ± 2.5 months.

The highest tertile of preprocedural fibrinogen levels was associated with increased age, BMI, female sex and diabetes mellitus. Regarding medication, patients in the highest tertile used more often ACE inhibitors and aspirin/acetylsalicylic acid (ASA). In the highest tertile, fewer patients were treated for a proximal LAD-lesion ($p=0.013$). With regard to biochemical characteristics, higher fibrinogen levels were associated with a higher ESR ($p<0.001$).

We found no significant association when we adjusted for the confounders from table 1, between fibrinogen and the -455G/A polymorphism ($p=0.664$). No statistically significant associations were observed between the -455G/A polymorphism and fibrinogen levels. Patients with the AA genotype had an average fibrinogen level of 3.53 g/l (SD 0.82) with median 3.3 (min-max: 2.6 - 5.6), for the AG genotype the average level was 3.80 g/l (SD 1.24), median 3.6 (min-max: 1.7-11.2), and for the GG genotype it was 3.76 g/l (SD 1.07) median 3.6 (min-max 0.4-8.1) ($p=0.65$). In the highest tertiles there were 40% A-carriers, 42% in the middle tertile and 41% of the patients were A-carriers in the lowest tertile ($p=0.79$).

Table 1. Baseline Characteristics of the total stented population and the subpopulation in tertiles of plasma fibrinogen

Baseline Characteristics	Total stented population	Preprocedural fibrinogen tertile (Range)			P for trend†
	(n=2,309)	<3.1 g/L (n=196)	3.2-4.0 g/L (n=225)	>4.1 g/L (n=202)	
Age, (y, SD)	61.9±10.7	61.1±10.1	61.1± 10.3	64.1± 11.3	0.005
BMI (kg/m ² , SD)	27.1±3.9	26.8± 3.7	26.8± 3.9	27.8± 4.4	0.010
Female Sex	653 (28%)	56 (29%)	50 (22%)	73 (36%)	0.007
Diabetes Mellitus	317 (14%)	29 (15%)	21 (9%)	44 (22%)	0.002
Current Smoking	577 (25%)	32 (16%)	49 (22%)	44 (22%)	0.30
Family History of MI	788 (34%)	69 (35%)	79 (35%)	75 (37%)	0.89
Previous MI	933 (40%)	79 (40%)	98 (44%)	98 (49%)	0.25
Previous PTCA	379 (16%)	43 (22%)	38 (17%)	33 (16%)	0.28
Previous CABG	285 (12%)	21 (11%)	32 (14%)	31 (15%)	0.38
Baseline Medication					
Beta-blockers	1,812 (78%)	159 (81%)	185 (82%)	156 (77%)	0.41
Ca-antagonists	1,180 (51%)	89 (45%)	104 (46%)	108 (54%)	0.20
Aspirin/ASA	1,940 (84%)	169 (86%)	187 (83%)	157 (77%)	0.042
ACE Inhibitors	463 (20%)	37 (19%)	61 (27%)	65 (32%)	0.01
Statins	1,267 (55%)	102 (52%)	135 (60%)	119 (59%)	0.22
Angiographic Data					
Total stent length	21.8±13.8	30.3± (17.6)	30.9 ± (16.5)	29.7± (17.9)	0.79
Proximal LAD	573 (25%)	67 (34%)	52 (23%)	46 (23%)	0.013
Biochemical data:					
ESR (mm/h): median (min – max)	11 (1-118)	6 (1-76)	10 (1-51)	23 (2-118)	<0.001

† *p*-value of the nonparametric Mann-Whitney test, or chi-square test, comparing the three fibrinogen tertile subgroups. SD, standard deviation, MI, myocardial infarction; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention; ASA, acetyl salicylic acid; ACE, angiotensin converting enzyme; LAD, left anterior descending coronary artery; ESR, erythrocyte sedimentation rate

Clinical follow-up

Major adverse cardiac events among the tertiles of fibrinogen levels and the total stented population during the follow-up period are listed in Table 2. Of the 2,309 patients, 203 (8.8%) had to undergo a TVR and 236 (10.2%) had combined endpoint. No association between the -455G/A polymorphism and TVR or combined endpoint was observed ($p=0.987$, $p=0.966$, respectively). Also in multivariable analysis, in which we adjusted for age, BMI, diabetes, hypertension, stent length, ESR, statin use and smoking, this polymorphism showed no association with TVR or combined endpoint ($p=0.845$, $p=0.858$, respectively). No increase in TVR (9.1%, 11.9%, 6.3%, $P=0.36$) and combined endpoint rate was observed across the tertiles (10.1%, 15%, 7.3%, $P=0.39$, respectively). Since statin use has been known to affect the role of inflammation, we stratified by statin use and looked at rates of TVR and combined endpoint across tertiles of fibrinogen levels. In our population statin use was neither associated with significant differences in rates of TVR ($p=0.62$, $p=0.82$, and $p=0.77$, respectively) nor with combined endpoint ($p=0.32$, $p=1.0$, and $p=1.0$, respectively) in tertiles of preprocedural levels of fibrinogen. There was no significant difference of fibrinogen levels and survival-free time for combined endpoint in the three tertiles.

Table 2. Major adverse cardiac events during follow-up

MACE	Preprocedural fibrinogen tertile (Range)				Total stented population
	<3.1g/L (n=196)	3.2-4.0 g/L (n=225)	>4.1 g/L (n=202)	P for trend	(n=2,309)
Cardiac death (%)	0 (0)	6 (3)	1 (1)	0.66	26 (1)
Death from other causes (%)	0 (0)	2 (1)	2 (1)	0.39	12 (0.5)
Myocardial Infarction (%)	1 (1)	2 (1)	4 (2)	0.14	15 (0.6)
TVR (%)	18 (9)	27 (12)	13 (6)	0.36	203 (9)
Combined endpoint† (%)	19 (10)	33 (15)	14 (7)	0.39	236 (10)

†Combined endpoint was defined as death presumably from cardiac causes, MI not attributable to another coronary artery than the target vessel, and TVR either by repeat PCI or CABG. MACE, major adverse cardiac events

Risk factors of restenosis and adverse cardiac event

For the multivariable Cox regression analysis for stent restenosis we included age, BMI, diabetes, hypertension, stent length, ESR, statin use and smoking. This analysis revealed that the risk of TVR and combined endpoint was not higher in patients in the second tertile for fibrinogen compared to the lowest tertile (RR=1.24, 95% C.I.=0.67 - 2.28 for TVR and RR=1.42, 95% C.I.=0.80 - 2.52 for combined endpoint). Comparison between the highest tertile for fibrinogen versus the lowest tertile showed no association as well (RR=0.60, 95% C.I.= 0.26-1.37 for TVR and RR=0.64, 95% C.I.= 0.29-1.44 for combined endpoint).

Discussion

In this population of patients that underwent coronary stent placement, the -455 G/A polymorphism and preprocedural fibrinogen levels were neither a risk factor for TVR nor for combined endpoint on follow-up.

The rare allele frequency of the polymorphism was in concordance with previous studies.^(12;20) Our results are in agreement with a smaller study performed by Völzke et al. who also tested the relationship between -455G/A polymorphism and PCI, only without stenting. This study of 511 patients found no association between this polymorphism and the risk of restenosis after PCI.⁽²¹⁾

Although it has been suggested that the -455G/A polymorphism could be linked to alterations in the functional properties of the fibrinogen protein, a study on fibrin clot structure *in vitro* has shown that it does not influence either the function or the structure of the protein.⁽²²⁾ The -455G/A polymorphism could have an effect on the regulation of the transcription of the gene. However, the association of the -455 G/A polymorphism of the beta-fibrinogen gene with plasma fibrinogen concentrations in patients with coronary artery disease is controversial. Some authors of smaller or comparable sized studies have found a positive association^(13;20;23;24), while others have not.⁽²⁵⁻²⁸⁾ In our large study we did not show that patients with the A allele had higher fibrinogen plasma levels. The usefulness of preprocedural fibrinogen levels to predict risk of restenosis after PCI is still disputed.⁽⁹⁻¹¹⁾ Although the role of inflammation in the development of atherosclerosis and its complications is firmly established, its role in the development of restenosis after PCI continues to be of interest.⁽²⁹⁻³¹⁾ In contrast with several older studies^(32;33), some recent studies have shown that

preprocedural levels of inflammatory markers such as C-reactive protein and interleukin-6 do not predict late coronary angiographic restenosis after elective stenting.^(30;34;35) The reason for this discrepancy may be the more intense use of statins and stents.

Discrepancies between our results and results obtained earlier may be at least partly explained by a greater prevalence of statin use in patients undergoing PCI. Statins have the capacity to attenuate inflammatory reactions after coronary stent implantation.^(29;36;37) However, in our population, the use of statins across tertiles of preprocedural fibrinogen did not affect rates of TVR or combined endpoint.

Another factor that might influence the evaluation of the role of inflammation in the restenosis process is time of sampling of inflammatory markers in relation to the time of intervention. Postprocedural plasma CRP concentration, measured 48 to 72 hours after PCI, has been shown to correlate more closely with restenosis than preprocedural plasma CRP values.⁽²⁹⁾ Furthermore, CRP levels after 48 hours have been associated with restenosis after carotid stenting as well.⁽³⁸⁾ In contrast, samples taken before the procedure were not predictive. Other inflammatory mediators in plasma, such as monocyte chemoattractant protein-1 also appear to predict restenosis if measured after, but not necessarily before PCI.⁽³⁹⁾ However, a marker can only be useful as a predictive factor if a preprocedural sample bares prognostic potential.

The relationship between inflammation and restenosis is complex.⁽⁴⁰⁾ Higher plasma levels of an inflammatory marker such as CRP, are associated with progression of CAD at areas remote from the stented lesion, and not necessarily with in-stent restenosis.⁽⁴¹⁾ Thus, studies that have examined a relationship between inflammatory markers and need for repeat revascularization (as opposed to target lesion revascularization specifically) may have overestimated the relationship between inflammatory markers and restenosis. At present, the most critical role of inflammatory markers lies in their ability to predict recurrent ischemic events and particularly mortality, rather than restenosis⁽⁴²⁾

Furthermore, we did not find a significant association between fibrinogen level and smoking status. This could be due to a lower percentage of smokers compared to previous studies or to a higher percentage of past smokers in our population.^(10;43) Since after smoking cessation, it may take as long as 20 years for the fibrinogen concentration to return to the level seen in nonsmokers.^(5;44)

Since our findings are non-significant, this inevitably raises the question of whether our sample was large enough to detect meaningful relative risk values.

As is indicated by the 95% confidence intervals (RR=1.24, 95% C.I.=0.67 – 2.28 for TVR) the overall sample size and the number of TVR-events in this study were large enough to confidently exclude that the relative risk for TVR of moderately increased fibrinogen is larger than 2.28.

Similarly, we can exclude that the relative risk (RR=0.60, 95% C.I.= 0.26-1.37) for TVR of severely increased fibrinogen is much larger than 1.37.

Conclusions

The -455 G/A polymorphism of the β -fibrinogen gene and preprocedural concentration of fibrinogen in plasma are not associated with the development of restenosis after an initially successful PCI procedure. Furthermore, preprocedural statin therapy did not influence the concentration of fibrinogen nor did it influence the incidence of combined endpoint. Nevertheless, this finding does not rule out that other polymorphisms in the β -fibrinogen gene could predict the development of restenosis. However, from our study we conclude that it seems unlikely that the fibrinogen pathway is an important factor in the development of restenosis after PCI. Genotyping of the -455 G/A polymorphism in the fibrinogen gene and preprocedural measurement of the concentration of fibrinogen in plasma in order to predict the risk of restenosis after stenting is not useful.

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