

Heme Oxygenase-1 Genotype and Restenosis After Balloon Angioplasty: A Novel Vascular Protective Factor

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OBJECTIVES	We investigated the association of the heme oxygenase-1 (HO-1) promoter genotype with the inflammatory response and restenosis after balloon angioplasty.
BACKGROUND	Heme oxygenase-1, which is induced by balloon angioplasty, can inhibit neointima formation and vascular remodeling. A dinucleotide repeat in the HO-1 gene promoter shows a length polymorphism that modulates HO-1 gene transcription. Short (<25 guanosine thymidine [GT]) repeats are associated with a 10-fold greater up-regulation of HO-1 than are longer repeats.
METHODS	We studied 381 consecutive patients who underwent femoropopliteal balloon angioplasty (n = 210) and comparison groups with femoropopliteal stenting (n = 68) and lower limb angiography (n = 103). C-reactive protein (CRP) was measured at baseline, 24, and 48 h. We evaluated patency at six months by duplex sonography and assessed the association of the length of GT repeats in the HO-1 gene promoter with postintervention CRP and restenosis.
RESULTS	Restenosis within six months was found in 74 patients (35%) after balloon angioplasty and in 21 patients (31%) after stenting. After balloon angioplasty, carriers of the short length (<25 GT) dinucleotide repeats had a lower postintervention CRP at 24 h (p = 0.009) and 48 h (p < 0.001) and a reduced risk for restenosis (adjusted relative risk 0.43, 95% confidence interval: 0.24 to 0.71, p < 0.001) compared with patients with longer alleles. After stenting or angiography, we found no association between the HO-1 genotype with CRP or restenosis.
CONCLUSIONS	The HO-1 promoter genotype that controls the degree of HO-1 up-regulation in response to stress stimuli is associated with the postintervention inflammatory response and the restenosis risk after balloon angioplasty. (J Am Coll Cardiol 2004;43:950–7) © 2004 by the American College of Cardiology Foundation

Understanding the inherited factors that influence a patient's susceptibility for restenosis after successful revascularization by femoropopliteal percutaneous transluminal angioplasty (PTA) may lead to better therapies (1–5). However, specific candidate genes have not yet been identified. Heme oxygenase-1 (HO-1) is a novel vascular protective factor with potent anti-inflammatory and anti-oxidant effects and the ability to inhibit the proliferation of smooth muscle cells (SMCs) and reduce negative vascular remodeling (6–11). Development of restenosis in large measure involves these very factors that are inhibited by HO-1: inflammation in the vessel wall, constrictive vascular remodeling, and hypertrophic neointima formation through SMC proliferation (12–14).

Heme oxygenase-1 is up-regulated by balloon angioplasty. However, humans may differ quantitatively in their ability to mount an HO-1 response. There is a length polymorphism in the form of a guanosine thymidine (GT)_n

dinucleotide repeat in the 5'-flanking region of the human HO-1 gene that modulates the quantitative level of HO-1 activity in response to a given stimulus (15,16). We hypothesized that the ability of a patient to respond strongly in terms of up-regulating HO-1 may be important in protection from restenosis.

Short (<25 GT) dinucleotide repeats in the HO-1 gene promoter show highly significant up-regulation of HO-1 to inflammatory stimuli; longer dinucleotide repeats, in contrast, have relatively low HO-1 inducibility (16). We previously found an association between short (GT)_n dinucleotide repeats (higher HO-1 induction) and a reduced rate of intermediate-term restenosis (7). However, the results of that study suffered from the fact that the study was a retrospective analysis, that few patients were evaluated, and that other factors that play a role in restenosis, such as the inflammatory response, were not evaluated. Thus, we present here a larger and prospective patient study. Given the known anti-inflammatory action of HO-1, we also asked whether an association exists between the HO-1 genotype and an attenuation of the inflammatory vessel wall response. Thus, the aims of the present prospective study were to assess whether carriers of short length (GT)_n dinucleotide repeats in the HO-1 gene promoter exhibit a lower postintervention inflammatory response measured by C-reactive protein (CRP) serum levels and a lower fre-

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Abbreviations and Acronyms

CI	= confidence interval
CO	= carbon monoxide
CRP	= C-reactive protein
GT	= guanosine thymidine
HO-1	= heme oxygenase-1
IQR	= interquartile range (range from the 25th to the 75th percentile)
PAD	= peripheral artery disease
PSV	= peak systolic velocity
PTA	= percutaneous transluminal angioplasty
RR	= risk ratio
SMC	= smooth muscle cell

quency of restenosis at six months after femoropopliteal balloon angioplasty. As comparison groups, we studied patients with peripheral artery disease who underwent femoropopliteal stenting or had a lower limb intra-arterial digital subtraction angiography without percutaneous intervention during the same time interval. The stenting group was included to comparatively study a mechanism of restenosis different from the mechanism arising after angioplasty; the angiography group was studied to assess whether the HO-1 promoter genotype is associated with the non-specific systemic inflammatory response after angiography.

METHODS

Study design. This study was designed as a prospective cohort study. We enrolled all consecutive inpatients with peripheral artery disease (PAD) Fontaine stage IIa, IIb, III, and IV who underwent primary successful femoropopliteal balloon angioplasty, femoropopliteal stenting, or lower limb angiography during a 12-month study period at the angiology department of a tertiary care university hospital. Patients who underwent primary thrombolysis were not eligible for the study. The study was approved by the local review board and ethics committee. All patients gave their written informed consent.

Definitions. The diagnosis of PAD was assessed by clinical evaluation, ankle brachial index measurements, and duplex sonography, and it was confirmed by lower limb angiography in all patients. Primary technical success was defined as a remaining diameter reduction <30% at the treated segment in the final angiogram. Residual stenosis in patients with primary technical success, indicating a successful but suboptimal result, was defined as a remaining absolute stenosis of 10% to 30% at the treated segment in the final angiogram. Poor run-off was defined as either occlusion or significant stenosis of the femoral or popliteal artery distal to the treated segment and/or in patients with occlusion or significant stenosis of at least two crural arteries. Restenosis was defined as $\geq 50\%$ diameter reduction at the dilated segment of the vessel within the first six months after PTA. Color-coded duplex sonography (5-MHz, linear array color probe [model XP 10; Acuson, Mountain View, California])

was used for categorization of restenosis (7,17). The peak systolic velocity (PSV) in the dilated region was determined and compared with the PSV in the preceding normal segment. A focal increase in the PSV of at least 140% (corresponding to a peak velocity ratio of ≥ 2.4) was considered indicative of a stenosis of $>50\%$ at that site (18).

Patient data. Two independent observers recorded patients' medical history and data from physical examination by a standard questionnaire at admission. Data were checked for inter-observer agreement at the day of patients' discharge; in case of discrepancies, the patient was re-evaluated by both investigators. Antecubital venous blood samples for determination of CRP were taken at baseline before the intervention, 24 h, and 48 h after the intervention. We used a high-sensitivity assay (N Latex CRP Mono, DADE Behring, Vienna, Austria) with a lower detection level of 0.03 mg/dl and a coefficient of variation of 4.6% for measurement of serum CRP levels.

Interventions. Two experienced interventionists performed all procedures after a standard protocol. Patients received 5,000 IU heparin intra-arterially after placement of the arterial sheath. We recorded the duration of fluoroscopy and dose of contrast agent (the nonionic, low-osmolality contrast agent Optiray 320 [Mallinckrodt, St. Louis, Missouri]). Location, degree, and length of the stenosis or occlusion as well as vessel size at the non-diseased segment proximal to the lesion were documented. The balloon diameter for subsequent angioplasty corresponded to the proximal non-diseased vessel diameter. Stent implantation (all self-expanding Easy Wallstents, Boston Scientific, Natick, Massachusetts) was performed as a bail-out procedure only in cases with a primary failure of balloon angioplasty due to a remaining absolute stenosis above 30% at the dilated segment. The technical results of PTA in terms of initial technical success, post-procedural residual stenosis, and number of run-off vessels were derived from the final angiograms.

One medical technical assistant performed color-coded duplex sonography, ankle brachial index, and oscillography 24 h after PTA for documentation of prolonged technical success or early restenosis. Peri-intervention and postintervention complications at the site of arterial puncture and at the dilated vessel segment were documented up to 48 h after the intervention. All patients received anti-thrombotic medication with acetyl salicylic acid 100 mg daily during the whole study period. Patients with stent implantation additionally received clopidogrel 75 mg daily for eight weeks postintervention, starting with a loading dose of 300 mg immediately after stent implantation. All patients routinely received once daily low dose low molecular weight heparin for three days starting 8 h postintervention. Glycoprotein IIb/IIIa antagonists were not given in any patient.

HO-1 genotype assessment. Genomic DNA was isolated from whole blood using standard techniques. Polymerase chain reaction amplifications of the HO-1 (GT)_n repeat length polymorphism was performed as described (7). Two

independent observers who were blinded with regard to patients' clinical data evaluated the dinucleotide repeat length. We divided allelic repeats into two subclasses after a classification based on transfection studies with low and high GT repeats (16): short repeats, with <25 (GT)_n were designated as allele class S (short), and longer repeats with ≥ 25 (GT)_n as allele class L (long).

Follow-up for restenosis. Patients were reinvestigated by six months after the procedure in the outpatient clinic to analyze the occurrence of restenosis: we performed duplex sonography, ankle brachial index, oscillography, evaluation of patient complaints, and physical re-examinations routinely in all patients. Ipsilateral follow-up angiograms for confirmation of the duplex findings were obtained in 132 patients (63%) after balloon angioplasty and in 45 patients after stent implantation (66%). Agreement for a $\geq 50\%$ restenosis was 92% between duplex sonography and angiography in the balloon angioplasty group and 93% in the stenting group. Two independent observers, who were blinded with regard to patients' HO-1 genotype, evaluated the follow-up data.

Statistical analysis. Continuous data are given as median and interquartile range (IQR from the 25th to the 75th percentile). Categorical data are given as counts and frequencies. Chi-square tests were used to compare groups of categorical data. The Mann-Whitney *U* test was used to compare unpaired continuous data. Friedman tests were used to analyze repetitive measurements of CRP levels. The median and its 95% confidence interval (CI) for serum CRP were calculated according to a standard formula (19). We used analysis of covariance to assess the association between HO-1 genotype and CRP levels at 24 and 48 h after balloon angioplasty and to account for the baseline value of CRP. As serum CRP was skewed to the right, we used log transformation to achieve a distribution resembling a normal distribution. The resulting effect size and the corresponding 95% CI was back-transformed. This means that the effect represents the ratio of serum CRP in non-carriers of the class S allele to serum CRP in carriers of the class S allele in the HO-1 gene promoter, clinically still a useful measure. Multivariate logistic regression analysis was applied to assess the independent effect of the HO-1 genotype (being a carrier of the class S allele) on six months patency while adjusting for the potentially confounding effects of other baseline variables. Baseline variables were selected for the model if they: 1) had either a clinically plausible relation with the outcome according to the TransAtlantic Inter-Society Consensus recommendations (1); or 2) appeared to be imbalanced between the HO-1 genotypes indicated by a value $p < 0.20$. We used a hierarchical modeling strategy to assess the effect of demographic variables and of procedure-related variables separately and jointly. As the outcome was a frequent event, we converted the odds ratios derived from the multivariate model to risk ratios (RR) and the 95% CI using a standard formula (20). A two-sided p value < 0.05 was considered as statistically significant. Calculations were

performed with Stata, release 7 (Stata, College Station, Texas), StatXact 5 (Cytel Software, Cambridge, Massachusetts) and SPSS for Windows (Version 10.0, SPSS Inc., Chicago, Illinois).

RESULTS

Study population. We evaluated 414 patients for the study within a 12-month period. Femoropopliteal balloon angioplasty was performed in 237 patients, femoropopliteal stenting in 71 patients, and lower limb angiography in 106 patients. In the balloon angioplasty group, the primary technical success rate was 96% ($n = 224$ of 237), 2 of 237 patients (1%) were lost to follow-up, and in 12 of 237 patients (5%), material for genetic analysis was missing. In the stenting group, the primary technical success rate was 100% ($n = 71$ of 71), 3 of 71 patients (4%) were lost to follow-up, and genetic data were complete. In the angiography group, 3 of 106 patients (3%) had missing material for genetic analysis. Therefore, we had to exclude 33 of 414 patients (8%) from the final analysis, which was based on data from 381 patients (92%): 210 patients in the balloon angioplasty group, 68 patients after stenting, and 103 patients with peripheral angiography. Median age in the balloon angioplasty, stenting, and angiography groups was 72 years (IQR, 63 to 78), 68 years (IQR, 57 to 74), and 67 years (IQR, 58 to 74), respectively, with 98 (47%), 42 (62%), and 65 (63%) males.

HO-1 genotype. Genotype frequencies of homozygous class S allele carriers (S/S), heterozygous class S allele carriers (S/L), and class S allele non-carriers (L/L) were 7% ($n = 14$), 47% ($n = 99$), 46% ($n = 97$) in the balloon angioplasty group, 7% ($n = 5$), 35% ($n = 24$), 57% ($n = 39$) in the stenting group, and 5% ($n = 5$), 43% ($n = 44$), 52% ($n = 54$) in the angiography group, respectively ($p = 0.37$). No significant differences of the class S allele frequency was observed between the groups of balloon angioplasty ($n = 113$, 54%), stenting ($n = 29$, 43%), and angiography ($n = 49$, 48%), respectively ($p = 0.23$).

Postintervention inflammatory response after balloon angioplasty. The CRP levels increased at 24 h and 48 h after balloon angioplasty ($p < 0.001$), indicating an acute inflammatory response. The HO-1 genotype was associated with postintervention CRP serum levels in the balloon angioplasty group: carriers of the class S alleles had lower postintervention CRP serum levels compared with non-carriers of the class S allele (Fig. 1). At 24 h, CRP levels of non-carriers of the class S allele were $1.46 \times$ the CRP level of class S allele carriers (95% CI: 1.14 to 1.88, $p = 0.009$) when adjusting for baseline CRP values; at 48 h, the baseline-adjusted ratio of CRP levels for non-carriers versus carriers of the class S allele was 1.70 (95% CI: 1.30 to 2.24, $p < 0.001$). We found no association between the HO-1 genotype and postintervention CRP levels in the comparison group after lower limb angiography (Fig. 1). Comparing the postintervention course of CRP in patients after angiog-

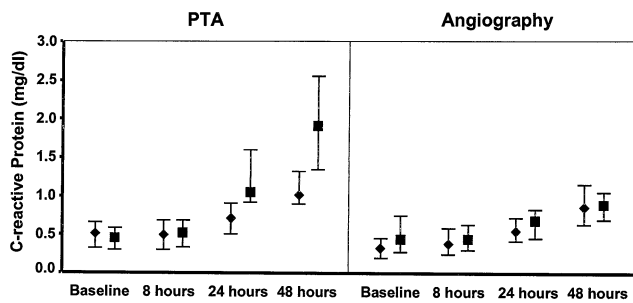


Figure 1. Course of C-reactive protein (median and 95% confidence interval of the median) from baseline to 48 h postintervention after femoropopliteal balloon angioplasty (n = 210) and after lower limb angiography (n = 103). Carriers and non-carriers of the class S allele in the heme oxygenase-1 (HO-1) gene promoter were compared. PTA = percutaneous transluminal angioplasty. **Diamonds** = carriers of the class S allele; **squares** = non-carriers of the class S allele.

raphy and PTA, patients after PTA and patients after angiography who were carriers of the class S allele showed a comparable CRP increase. In contrast, non-carriers of the class S allele exhibited a significantly higher CRP increase after PTA than did carriers of the class S allele and patients after angiography (Fig. 1).

Postintervention inflammatory response after stenting. C-reactive protein levels increased at 24 h and 48 h after stenting (p < 0.001), also indicating an acute inflammatory response after the procedure. However, no significant association between the HO-1 genotype and postintervention CRP was found at 24 h (p = 0.70) or 48 h (p = 0.31).

Restenosis after balloon angioplasty. Seventy-four patients (35%) after balloon angioplasty had restenosis of the treated vessel segment by six months after presentation. Patients with restenosis had higher postintervention CRP levels at 24 h (1.26 mg/dl; IQR, 0.77 to 2.26) and 48 h (2.06 mg/dl; IQR, 1.06 to 3.69) compared with patients without restenosis (24 h 0.80 mg/dl, IQR 0.34 to 1.75, p < 0.001; 48 h 0.97, IQR 0.64 to 2.08, p < 0.001).

The allele frequencies of the (GT)_n microsatellite in the HO-1 promoter region of patients with and without restenosis are shown in Figure 2. The HO-1 genotype was associated with the occurrence of restenosis; 14 patients who were homozygous class S allele carriers had the lowest rate of restenosis, whereas heterozygous class S patients had a gradually increased restenosis rate, and homozygous L carriers exhibited the highest recurrence rates (Fig. 3).

We performed an univariate comparison between carriers and non-carriers of the class S allele to identify possible confounding factors (Table 1). Due to the small number of homozygous class S allele carriers, homozygous and heterozygous patients were grouped and analyzed together. Demographic data and clinical characteristics were highly similar between class S carriers and non-carriers, although carriers of the class S allele were more frequently smokers, and non-carriers had a slightly higher incidence of coronary artery disease. The procedure-related variables: "length of the treated lesion" and "poor run-off" also showed a trend towards a difference between the two groups (Table 1). We

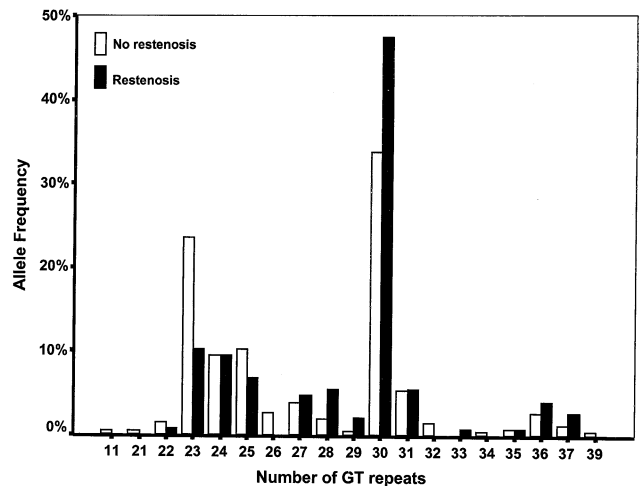


Figure 2. Frequency distribution of the guanine thymidine (GT)_n repeats in patients with and without restenosis after femoropopliteal balloon angioplasty (n = 210): lower number of (GT)_n repeats in patients without restenosis compared with patients with restenosis (p = 0.001).

then applied a multivariate logistic regression model to assess the effect of the HO-1 genotype on patency by six months after PTA adjusting for potentially confounding effects. Carriers of the class S allele in the HO-1 gene promoter had a very significantly reduced risk for restenosis at six months (relative risk 0.43, 95% CI: 0.24 to 0.71, p < 0.001) adjusting for sex, age (years), smoking (yes vs. no), diabetes mellitus (yes vs. no), Fontaine stage (IIa to IV), length of lesion (quartiles), residual stenosis after PTA (yes vs. no), poor run-off (yes vs. no), and intimal dissection (yes vs. no) (Table 2).

To evaluate the extent to which CRP levels are an intermediate on the association between the HO-1 genotype and restenosis within six months, we further calculated two fully adjusted models including CRP values at 24 or 48 h (quartiles). The association between the HO-1 gene polymorphism and restenosis was slightly attenuated when

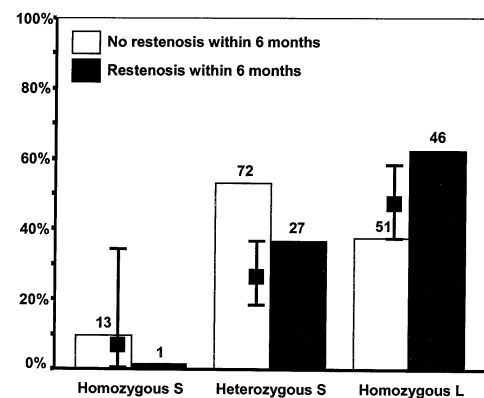


Figure 3. Lower restenosis rates after femoropopliteal balloon angioplasty in homozygous and heterozygous class S allele carriers compared with non-carriers of the class S allele in the heme oxygenase-1 gene promoter (p < 0.001). **Bars** indicate percentage of patients with and without restenosis according to the heme oxygenase-1 (HO-1) promoter genotype; point estimates and 95% confidence interval indicate the percentage of patients with restenosis for each HO-1 promoter genotype.

Table 1. Comparison of Carriers Versus Non-Carriers of the Class S Allele in the Heme Oxygenase-1 Gene Promoter in 210 Patients After Femoropopliteal Balloon Angioplasty

	Carriers of the Class S Allele (n = 113)	Non-Carriers of the Class S Allele (n = 97)	p Value
Median age in yrs (IQR)	71 (61 to 78)	73 (66 to 78)	0.37
Male gender	66 (58%)	46 (47%)	0.11
Arterial hypertension	91 (81%)	70 (72%)	0.19
Hyperlipidemia	94 (83%)	74 (76%)	0.21
Smoking	39 (35%)	22 (23%)	0.060
Diabetes mellitus	51 (45%)	48 (50%)	0.53
Coronary artery disease	52 (46%)	54 (56%)	0.17
Carotid stenosis \geq 25%	63 (56%)	50 (52%)	0.54
Fontaine stage of PAD			0.63
IIa	8 (7%)	6 (6%)	
IIb	69 (61%)	65 (67%)	
III	14 (12%)	7 (7%)	
IV	22 (20%)	19 (20%)	
Recurrent stenosis after prior PTA	29 (26%)	33 (34%)	0.17
Complete vessel occlusion	27 (24%)	24 (25%)	0.89
Median grade of stenosis in % (IQR)	90 (80 to 95)	90 (80 to 99)	0.38
Median length of lesion in mm (IQR)	60 (30 to 90)	40 (30 to 80)	0.091
Vessel size proximal to the lesion in mm (IQR)	5 (4 to 6)	5 (4 to 6)	0.86
Residual stenosis	61 (54%)	57 (59%)	0.49
Poor run-off	16 (14%)	23 (24%)	0.076
Initial dissection	20 (18%)	18 (19%)	0.87

IQR = interquartile range; PAD = peripheral artery disease; PTA = percutaneous transluminal angioplasty.

entering CRP levels measured at 24 h to the final model (RR 0.45, 95% CI: 0.24 to 0.76, $p = 0.001$) and was further reduced when using the 48 h CRP values (relative risk 0.53, 95% CI: 0.28 to 0.89, $p = 0.012$). However, because the HO-1 polymorphism certainly affects the risk of restenosis through multiple inflammatory mechanisms, the attenuation was not complete.

Restenosis after stenting. In-stent restenosis within six months was found in 21 of 68 patients (31%). We found no association between the HO-1 genotype and six months restenosis after femoropopliteal stenting: class S carriers had a similar rate of in-stent restenosis compared with non-carriers of the class S allele (8 of 29, 28% vs. 13 of 39, 33%, $p = 0.61$).

DISCUSSION

We found that the HO-1 promoter genotype was associated with the occurrence of restenosis after femoropopliteal balloon angioplasty. Patients with short repeats [S allele: <25 (GT)_n] in the HO-1 gene promoter exhibited a lower postintervention inflammatory response and a lower restenosis rate at six months compared with patients with longer [L alleles: >25 (GT)_n] repeats. This suggests that a stronger HO-1 response, which is associated with the short GT repeats, is protective against restenosis as compared with the lower HO-1 response in the absence of the class S allele. Modulation of the postintervention vascular inflammatory response seems to be an underlying mechanism of the

Table 2. Logistic Regression Model Assessing the Independent Association of the Heme Oxygenase-1 Genotype "Carrier of the Class S Allele" and Restenosis at Six Months in 210 Patients After Femoropopliteal Balloon Angioplasty

	Risk Ratio	95% Confidence Interval	p Value
Model I (univariate)			
Carriers of the class S allele	0.52	0.33-0.78	0.001
Model II (adjusted for demographic baseline variables*)			
Carriers of the class S allele	0.47	0.28-0.74	<0.001
Model III (adjusted for procedure-related variables†)			
Carriers of the class S allele	0.48	0.28-0.76	0.001
Model IV (adjusted for demographic and procedure-related variables‡)			
Carriers of the class S allele	0.43	0.24-0.71	<0.001

Class S allele denotes <25 (GT)_n repeats in the heme oxygenase-1 gene promoter. *Gender, age (yrs), diabetes mellitus (yes vs. no), smoking (yes vs. no), Fontaine stage (IIa to IV); †length of lesion (quartiles), residual stenosis (yes vs. no), poor run-off (yes vs. no), initial dissection (yes vs. no); ‡variables of model II and model III.

beneficial effects of HO-1 up-regulation after balloon angioplasty. These findings in the present patient sample are in concert with former preliminary and retrospective data in a smaller patient sample from our group (7) and suggest that the HO-1 functional polymorphism may have an important influence on the pathophysiology of restenosis after balloon angioplasty.

Heme oxygenase is the rate-limiting enzyme in the catabolism of heme into biliverdin (which is rapidly converted to bilirubin), free iron (which induces the up-regulation of ferritin), and CO. Vascular SMCs and endothelial cells can express HO-1, the inducible isoform of HO, which has potent anti-inflammatory and anti-oxidant capacity (21). Heme oxygenase-1 exerts its protective effects via the products generated after the action of HO-1 on heme. All three products can be protective. Carbon monoxide exerts potent anti-proliferative (of SMCs) and anti-inflammatory effects (of endothelial cells) in the vascular wall and, thereby, influences neointima formation, SMC activation, and vascular remodeling (21-25). In addition, CO suppresses the pro-inflammatory while boosting the anti-inflammatory response of monocytes to lipopolysaccharide (21). Ferritin has a potent anti-apoptotic effect in endothelial cells as well as protecting cells from the injurious effects of free iron. Bilirubin is a known anti-oxidant that also has anti-apoptotic effects.

Balloon angioplasty induces vascular injury manifested by an inflammatory response and subsequent cell proliferation of vascular SMCs (26-30). The postintervention course of acute phase reactants reflects the extent of vascular inflammation at the site of the treated segment after percutaneous catheter interventions. In particular, serum CRP is a sensitive, specific, and fast-reacting marker of the acute phase reaction that provides an indirect measure of the cytokine-dependent inflammatory process in the arterial wall (31). Higher CRP levels indicate enhanced vascular inflammation. In this context, CRP was shown to predict restenosis after femoropopliteal PTA (32). Consistently, in the present study, patients who experienced restenosis had higher postintervention CRP levels compared with patients with patent vessels at six months. Both damage to endothelial cells during balloon dilation and potentially physical strain on the vessel wall likely modulate the level of gene expression in activated vascular SMCs and endothelial cells and amplify the proliferative phase of vessel repair (33). A protective response by means of up-regulation of genes that suppress the pro-inflammatory process after endothelial injury has been suggested earlier (34,35). Heme oxygenase-1 has been a prime candidate for a vascular protective gene in this regard (6,7,35,36).

A (GT)_n dinucleotide repeat in the 5' flanking region of the human HO-1 gene is highly polymorphic and modulates HO-1 gene expression (16,37). A major strength of the present study is its confirmation that the 5'-flanking polymorphism in the HO-1 gene is significantly associated with the restenosis after PTA in a prospective study on an

independent patient sample and its demonstration of an association between CRP levels as a measure of postintervention inflammatory response and the HO-1 genotype. These findings, which are in concert with experimental findings in rodents in which HO-1 is induced and shown to protect from intimal hyperplasia, strongly support HO-1 as a relevant factor in the pathogenesis of restenosis and as an anti-inflammatory factor in general. The differential induction of HO-1, which is almost certainly correlated with differential production of CO and the other products of HO-1 action on heme, may account for the inhibition of excessive proliferation of vascular SMCs, constrictive neointimal hyperplasia, and recurrent lumen narrowing due to negative vascular remodeling (6,38). Nevertheless, it cannot be ruled out that mechanisms other than an anti-inflammatory action of HO-1 are operative as well, like a direct anti-proliferative effect of CO on vascular SMCs.

Cautious interpretation of the present findings suggests an additive or "gene-dose" effect of the HO-1 gene polymorphism; 14 patients who were homozygous class S allele carriers had the lowest rate of restenosis, whereas heterozygous class S patients had a gradually increased restenosis rate, and homozygous L carriers exhibited the highest rates of recurrences (Fig. 3).

In patients with stent implantation, no association between the HO-1 genotype, vascular inflammation, and restenosis was found. Restenosis after stenting is mainly due to in-stent neointimal hyperplasia (39,40), whereas, after balloon angioplasty, negative vascular remodeling plays a major role in the development of restenosis. This suggests that the mechanism responsible for the beneficial effect of HO-1 after balloon angioplasty is due to an attenuation of negative remodeling rather than an inhibition of neointimal hyperplasia, otherwise one would expect an association of the HO-1 genotype also with in-stent restenosis. However, to confirm this hypothesis, data from intravascular ultrasound observations would be needed, comparing the mechanisms of restenosis after balloon angioplasty and stenting in different HO-1 genotypes.

Restenosis occurs in up to 60% of patients within the first year after femoropopliteal interventions with considerable morbidity and costs (1,7). With increasing numbers of procedures performed in any given patient, late sequelae and the need for costly re-interventions become more frequent (1,2). Identification of this novel genetic risk factor may help to predict the risk of restenosis after balloon angioplasty. Determination of the HO-1 genotype before a scheduled intervention may facilitate a targeted use of adjunctive measures for prevention of restenosis-like intraluminal brachytherapy or the recently introduced drug-coated stents (17,41-43). Furthermore, the association between HO-1 genotype and inflammatory response after balloon angioplasty suggests that therapeutic benefit might also derive from the use of high-dose anti-inflammatory medications after the procedure in persons who are genetically susceptible.

Whereas it might be worth evaluating adeno-associated virus or adenovirus-mediated delivery of the HO-1 gene, which has been successful in animal models (44,45) in patients undergoing balloon angioplasty, a more practical and likely clinically acceptable approach would be induction of HO-1 with a substance that is accepted for clinical use. Induced expression of HO-1 suppresses restenosis (6,8). While induction of HO-1 is an appealing potential approach to prevent restenosis, our findings here point out the possible difficulty in patients carrying the low HO-1 response genotype. In those cases, one may have to devise methods of overcoming the low responsiveness; alternatively, one might use one or more of the products produced by HO-1 action on heme such as CO to prevent restenosis (38). Administration of low doses of CO by inhalation very markedly suppresses restenosis. Administration of 250 parts per million (ppm) for only a single hour before angioplasty was highly effective at reducing the amount of neointimal proliferation in rats as measured on day 14 postintervention (38). Other products of HO-1 action on heme, such as ferritin, biliverdin (46), and bilirubin also deserve evaluation for their effects.

Study limitations. The association we have shown between short repeats of the HO-1 gene promoter polymorphism and reduced risk of restenosis, as well as the association between short repeats and reduced levels of CRP after angioplasty, is strong evidence that the association is "real." Nevertheless, the issue of whether this polymorphism indeed results in a functional change, or whether it is linked to a gene that exerts the true functional change, remains unresolved. However, several *in vitro* and *in vivo* studies have shown directly that HO-1 has benefits on processes such as those assayed in this study and have demonstrated the functional importance of this polymorphism; given the combined data, we believe that our hypothesis is strongly supported (16,38). We do not believe that selection bias plays a major role, because genetic data was available on approximately 95% of patients. Patients with missing genetic data were comparable to the remaining patients (data not shown). Information bias is unlikely because outcome assessors were blinded to the HO-1 genotype status. Further, our study is necessarily of an observational nature. Accordingly, our results may be explained by confounding. Therefore, we tried to control for baseline imbalances (Table 1) by multivariate modeling (Table 2). The possibility of residual or undetected confounding is small but cannot be ruled out completely.

Conclusions. The heme oxygenase-1 gene promoter polymorphism is associated with the risk of restenosis six months after femoropopliteal balloon angioplasty but not after stent implantation. Patients after balloon angioplasty with the short (GT)_n repeats, which are indicative of a strong HO-1 response, exhibit a lower postintervention inflammatory response, as reflected in increases in CRP and a reduced rate of restenosis. The production of carbon monoxide consequent to HO-1 induction may inhibit

vascular inflammation, SMC proliferation, and constrictive vascular remodeling in humans, as has been demonstrated in experimental animals.

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