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

Monraats, P.S.

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Inflammation and apoptosis genes and the risk of restenosis after percutaneous coronary intervention

Pascalle S. Monraats, Florentine de Vries, Laura W. de Jong, Douwe Pons, Var sha D.K.D. Sewgobind, Aeilko H. Zwinderman, Moniek P.M. de Maat, Leen M. 't Hart, Pieter A. Doevendans, Robbert J. de Winter, René A. Tio, Johannes Waltenberger, Rune R. Frants, Arnoud van der Laarse, Ernst E. van der Wall, J. Wouter Jukema

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Abstract

Background

Genetic factors appear to be important in the development of restenosis after PCI, as well as in the process of inflammation, a pivotal factor in restenosis. Caspase-1, IL-1-receptor and PTPN22 are important mediators in the inflamma tory response and caspase-1 also in apoptosis. Therefore, we examined whether polymorphisms in these candidate genes are related to the risk of developing restenosis after PCI.

Methods

The GENetic Determinants of Restenosis (GENDER)-project is a multicenter prospective follow-up study. The 5352G/A (L235L) caspase-1-polymorphism, the 7464C/G (A124G) IL-1r-polymorphism, and the 1858C/T (R620W) PTPN22-polymorphism were genotyped. To examine the functional effect of the caspase-1 polymorphism, mature plasma IL-1β levels were measured by ELISA in LPS-stimulated whole blood from a subpopulation of patients.

Results

3,104 patients, age 62.1±10.7 years, were included after successful PCI. A signifi cant association between the 5352AA genotype of the caspase-1 gene and TVR (RR 2.2, 95% CI 1.32-3.76) was observed after correcting for clinical variables. An giographic analysis of a subgroup of patients (N=478) also showed an increased risk for developing restenosis for patients having the 5352GA/AA genotype (p=0.001). The results were corroborated, however, not statistically significant by somewhat higher mature IL-1β levels in patients with the 5352AA genotype.

Conclusions

The present study shows that patients with the 5352AA genotype in the caspase-1 gene are at increased risk to develop restenosis. If confirmed by other studies, screening patients for this genotype can lead to better risk stratification and provide indications for improving individual treatment, for instance by provid ing a new target for drug-eluting stents.

Introduction

Percutaneous coronary intervention (PCI) has become the main treatment for atherosclerotic lesions. However, still an important limitation of this procedure is the occurrence of restenosis.[1] Treatment with drug eluting stents (DES) has reduced the restenosis rate, however they do not solve the renarrowing prob lem entirely. Furthermore long-term experience with DES in coronary arteries is awaited. Therefore, stratification of patients at increased risk of developing restenosis and finding the right drug target can be useful and lead to improved individual therapy.

Restenosis is not a random event, but it affects selectively a certain subset of pa tients who are prone to develop lumen renarrowing after PCI. Ample evidence indicates that inherited factors may explain at least part of the risk of resteno sis in certain patients, since it cannot be attributed only to conventional clini cal variables.[2] Inflammatory responsiveness, resulting in neointimal formation, plays a pivotal role in the process of restenosis.[3] Several inflammatory genes have already been reported to be associated with the development of resteno sis.[4-7] These data provoked further research on the development of restenosis in relation with three other key regulators of the immune system. Recent studies revealed that caspase-1 (also known as interleukin-1β converting enzyme/ICE), interleukin-1 receptor 1 (IL-1r) and protein tyrosine phosphatase non-receptor type 22 (PTPN22) are such important mediators in the inflammatory response.[8-10]

Caspase-1 is involved in cytokine maturation of IL-1β, IL-1α and IL-18. Further more, it is involved in apoptosis. IL-1 is a potent pro-inflammatory cytokine that occurs as IL-1α and IL 1β. The biological activity of IL-1α and IL 1β is initiated by binding with the IL-1r and is inhibited by IL-1 receptor antagonist (ILRa).[8] IL-1 has been shown to be a significant determinant of intimal hyperplasia and has been demonstrated to stimulate the thrombogenic response in endothelial cells as well as the production of endothelial-derived growth factor.5 PTPN22, encodes for lymphoid protein tyrosine kinase (LYP), which is a key molecule regulating T cell receptor-signaling in memory/effector T lymphocytes. It is im portant in negative control of T-cell activation and in T-cell development.[9] The Arg620Trp substitution, which we examined in this study, has been found to encode an important functional change, such that the tryptophan-bearing LYP allele cannot associate with the C-terminal src kinase (Csk), a partner molecule in an inhibitory complex that regulates key T cell receptor signaling kinases (Lck, Fyn, ZAP-70).[10] Furthermore, several studies have demonstrated that this poly

morphism plays a pivotal role in a wide spectrum of inflammatory disorders as systemic lupus erythematosus, rheumatoid arthritis and diabetes.[11-13] Due to potent (pro-) inflammatory actions of caspase-1, IL-1r and PTPN22, these three substances were suspected to influence the occurrence of restenosis after PCI. Therefore the aim of this study was to assess whether polymorphisms in the genes encoding caspase-1, IL-1r and PTPN22 are related to the risk of develop ing restenosis after PCI. Furthermore, we performed whole blood stimulation analysis in a subpopulation of patients, to further increase our understanding of the function of the polymorphisms that are associated significantly with reste nosis.

Methods

GENetic DEterminants of Restenosis (GENDER) project

Study design

The present study population has been described previously.[14] In brief, the GE Netic DEterminants of Restenosis (GENDER) project was designed to study the association between various gene polymorphisms and clinical restenosis, defined by target vessel revascularization (TVR). Patients eligible for inclusion were treated successfully for stable angina, non-ST-elevation acute coronary syndromes or silent ischemia by PCI in four out of 13-referral centers for inter ventional cardiology in the Netherlands (Academic Medical Center Amsterdam, University Medical Center Groningen, Leiden University Medical Center and Academic Hospital Maastricht). Patients treated for acute ST elevation myocar dial infarction were excluded. Also excluded from analysis were patients suffer ing from events occurring within one month from PCI, since these events were considered attributable to sub-acute stent thrombosis or occluding dissections, and not to restenosis per se.

PCI procedure

Experienced operators, using a radial or femoral approach, performed standard angioplasty and stent placement. Before the procedure, patients received aspirin 300 mg and heparin 7500 IU. The use of intracoronary stents and additional medication, such as glycoprotein IIb/IIIa inhibitors, were carried out at dis cretion of the operator. In case a stent was implanted, patients received either ticlopidin or clopidogrel for at least one month following the procedure depend ing on local practice. During the study, no drug-eluting stents were used.

Follow-up and study endpoints

Follow-up lasted for at least nine months, except when a coronary event oc curred. Patients were either seen in outpatient clinics or contacted by telephone. TVR, either by PCI or coronary artery bypass grafting (CABG), was designated the primary endpoint, since it is considered most relevant by regulatory agen cies. An independent clinical events committee evaluated the clinical events.
The study protocol meets the criteria of the Declaration of Helsinki and was approved by the Medical Ethics Committees of each participating institution. Written informed consent was obtained from all participating patients prior to the PCI procedure.

Genetic methodology

Blood was collected in EDTA tubes at baseline and genomic DNA was extracted following standard procedures. In this population we determined genotypes of the following polymorphisms: caspase-1 5352G/A (L235L, rs580253), IL-1r 7464C/G (A124G, rs2228139) and PTPN22 1858C/T (R620W, rs2476601). The caspase-1 and IL-1r polymorphisms were selected with the SNPper database (www.snpper.chip.org). Criteria used for selection of the polymorphisms, were a possible func tional effect (exon-polymorphism with when possible an amino acid substitu tion). Furthermore, the polymorphism had to be validated and needed to have a known frequency of >5% determined in a Caucasian population. The functional effect of these polymorphisms has not been described. The PTPN22 polymor phism we determined was selected based on literature reports, in which the functional effect of this polymorphism has already been well described. Previ ous studies show that the 1858C/T substitution disrupts an interaction between LYP and the protein tyrosine kinase Csk. Normally the interaction between LYP and Csk leads to the inhibition of T-cell activation. The 1858T allele may translate biologically to the potential for hyper-reactive pathogenic T-cell re sponse.[11-13] To determine the different polymorphisms, two techniques were used. The first method for genotyping used a MassArray platform according to manufacturers' protocols. A multiplex assay was designed using Assay designer software (Sequenom, Hamburg, Germany). All PCR reactions had a final vol ume of 5 µL and contained standard reagents and 2.5 ng of genomic DNA. The primer sequences used for PCR for the caspase-1 polymorphism are: Forward: 5'ACGTTGGATGAAACACTCTGAGCAAGTCCC3' and Reverse: 5'AC GTTGGATGCAAACTTGGGCAGTTCTTGG3', and for the IL-1r polymor phism: Forward: 5' ACGTTGGATGGTGCAAAATTTGTGGAGAATG3' and Reverse: 5' ACGTTGGATGCTCCTGCAACGGGTAGTTTC3'. After PCR a primer extension reaction was performed to introduce mass-differences between

alleles and, after removing salts by adding a resin, ~15 nL of the product was spotted onto a target chip with 384 patches containing matrix. Mass differences were detected using an Autoflex (Bruker, Wormer, Netherlands) matrix-assisted laser desorption\ionistation time-of-flight mass spectrometry (MALDI-TOF) and genotypes were assigned real-time using Typer 3.1 software (Sequenom). As quality controls, 5-10% of the samples were genotyped in duplicate. No inconsis tencies were observed. Cluster plots were made of the signals from the low and the high mass allele. Two independent researchers carried out scoring. Disagree ments or vaguely positioned dots (<1%) produced by Genotyper 3.0 (Sequenom) were left out of the results.

To assess the 1858C/T polymorphism of the PTPN22 gene we made use of Taqman analysis. The 5'nuclease, or Taqman, PCR assay has been described previously.[15] In brief, a fluorigenic probe, consisting of an oligonucleotide labelled with both a fluorescent reporter dye and a quencher dye, was included in a typical polymerase chain reaction. Probes specific for both alleles (C/T) and labelled with a differ ent fluorescent reporter dye were included in the PCR assay. Sequences of the primers used are; Forward: 5'CCAGCTTCCTCAACCACAATAAATG3' and Reverse: 5'CAACTGCTCCAAGGATAGATGATGA3'. Cycle conditions were: 95oC for 10 min, followed by 15 sec on 92oC, followed by 1 min at 60oC. The last two steps were repeated 40 times. Fluorescence measurements were made after the PCR on the ABI PRISM 7200 or 7700 Sequence Detection System (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). This systems' software au tomatically processes the fluorescence data and shows a plot on which each dot represents a particular sample. To confirm genotype assignments the procedure was repeated in 10% of the samples. The operators who performed genotype determinations were unaware of the patients' clinical characteristics. Here also, two independent researchers carried out scoring. Disagreements or vaguely po sitioned dots (<1%) produced by ABI PRISM 7700 were left out of the scoring.

In vitro challenge of whole blood by LPS

Caspase-1 is a converting enzyme of pro-IL-1β and IL 1β is a cytokine, which stimulates the inflammatory response when activated by caspase-1. Therefore, we determined mature plasma IL-1β levels to examine the functional effect of the 5352G/A caspase-1 polymorphism. We hypothesized that patients with the AA genotype of the capsase-1 gene, who showed a higher risk of developing re stenosis, would demonstrate an increased inflammatory response and therefore higher levels of IL-1β. We performed whole blood stimulation by lipopolysac charide (LPS) in a subpopulation (N=69) of the GENDER patients, treated in the Leiden University Medical Center. The subpopulation was selected to have

an equal number of patients for each genotype-group of the caspase-1 gene; furthermore patients were matched for gender and age. Venous blood was drawn from this subset of patients into BD Vacutainer Heparin tubes (BD Alphen aan den Rijn, The Netherlands). Blood samples were collected and were diluted 1:1 with RPMI (Invitrogen, Breda, The Netherlands), followed by incubation with LPS (Escherichia coli L2630, Sigma, Uithoorn, The Netherlands) at a concentration of 1, 10 and 50 ng/μL. Baseline cytokine concentrations were measured under similar conditions in the absence of LPS challenge. After 24 h of incubation at 37 °C in a CO_2 incubator, plasma was prepared by centrifugation at 1800g for 10 min at 4 °C. Plasma samples were stored at -80 °C until assays were performed. Cytokine concentration of IL-1β was measured by a commercial enzyme-linked immunosorbent assay (ELISA)-kit (Pelikine™, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service "Sanquin", Leiden, The Netherlands). The coefficient of variation (CV) was 10.5% over a broad range. Measurements were performed in duplicate, and the mean value of two measurements was used. If the difference between the duplicates was >10.5%, the analysis was repeated.

Angiographic assessment

Quantitative computer-assisted angiographic analysis was performed off-line on angiograms obtained just before, immediately after stenting, and at follow-up in a subpopulation of patients from the GENDER-study, who were scheduled for re-angiography at six-months, according to previously described standard procedures.[16] Identical projections were used for all angiograms. Binary restenosis was defined as a stenosis diameter >50% within the stent or in the 5-mm segments proximal or distal to the stent at follow-up angiography.[17] Quantitative analysis of angiograms was performed by operators not involved in the stenting procedure and unaware of the genetic data (Heartcore, Leiden, the Netherlands).

Statistical analysis

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 frequencies were determined by gene counting, the 95% confidence intervals of the allele frequencies were calculated from sample allele frequencies, based on the approximation of the binominal and normal distributions in large sample sizes.

Continuous variables are expressed as mean ± standard deviation and were compared by means of the unpaired, two-sided t test. Discrete variables are expressed as counts or percentages and were compared with the Chi-squared test. In the

first stage, the association between caspase-1, IL-1r and PTPN22 polymorphisms and TVR was assessed using the Cox proportional regression model under a co-dominant genetic model. No adjustments for covariates were performed at this stage so that we could assess their possible involvement in the causal pathway. If less than 10 patients were homozygous for a particular allele, two groups were formed with the absence or presence of that allele as group variable.
All polymorphisms were also assessed using dominant and recessive models, and the model with the lowest Akaike information criteria was used in multivariable regression analysis. 18 Multivariable regression analysis of the TVR risk was per formed with all polymorphisms, using a stepwise backward selection algorithm. In the final step clinical variables associated with TVR, also including age and gender, were entered into the regression model. The results of the IL-1β plasma levels are expressed as median with interquartile range. Differences in IL-1β pro duction between groups were examined using the Kruskal-Wallis test.
A p value <0.05 was considered statistically significant. Statistical analysis was carried out using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

Results

A total of 3,146 patients had a complete follow-up (99.3%) with a median dura tion of 9.6 months (interquartile range 3.9). Out of 3,146 patients 42 had an event in the first 30 days. These patients were excluded from further analysis, accord ing to the protocol. The remaining 3,104 patients had a mean age of 62.1 ± 10.7 years. Of the patients 888 (28.6%) were female, and 453 (14.6%) had diabetes mellitus. 812 patients (26.2%) received glycoprotein IIb/IIIa inhibitors. Stents were used in 2,309 (74.4%) patients. A total of 4,061 lesions were treated in this unselected patient sample. Complex (type C) lesions, classified according to the modified American College of Cardiology and American Heart Association Task Force classification, were treated in 802 patients (25.8%). Other patient charac teristics, as well as details of the interventions are summarized in table 1.

Table 1. Demographic, clinical and lesion characteristics of 3,104 patients with and without TVR after one-month follow-up

	Patients with TVR (n=304)	Patients without TVR (n=2,800)	Total (n=3,104)
Age (years)	61.7 ± 10.1	62.2 ± 10.8	62.1 ± 10.7
BMI ($kg.m^{-2}$)	26.9 ± 3.7	27.0 ± 3.9	27.0 ± 3.9
Male sex	220 (72.4%)	1,996 (71.3%)	2,216 (71.4 %)
Diabetes	63 (20.7%)	390 (13.9%)	453 (14.6%)
Hypercholesterolemia	188 (61.8%)	1,702 (60.8%)	1,890 (60.9%)
Hypertension	138 (45.4%)	1,121 (40.0%)	1,259 (40.6%)
Current smoker	62 (20.4%)	700 (25.0%)	762 (24.5%)
Family history of MI	121 (39.8%)	977 (34.9%)	1,098 (35.4%)
Previous MI	109 (35.9%)	1,130 (40.4%)	1,239 (39.9%)
Previous PCI	64 (21.1%)	493 (17.6%)	557 (17.9%)
Previous CABG	36 (11.8%)	340 (12.1%)	376 (12.1%)
Stable angina	198 (65.1%)	1,881 (67.2%)	2,079 (67.0%)
Multivessel disease	148 (48.7%)	1,284 (45.9%)	1,432 (46.1%)
Peripheral vessel disease	12 (3.9%)	92 (3.3%)	104 (3.4%)
Lipid lowering medication	171 (56.3%)	1,516 (54.1%)	1,687 (54.3%)
Restenotic lesions	27 (8.9%)	181 (6.5%)	208 (6.7%)
Total occlusions	56 (18.4%)	372 (13.3%)	428 (13.8%)
Type C lesion	94 (30.9%)	708 (25.3%)	802 (25.8%)
Proximal LAD	70 (23.0%)	619 (22.1%)	689 (22.2%)
RCX	75 (24.7%)	764 (27.3%)	839 (27.0%)

BMI: body mass index, MI: myocardial infarction, LAD: left anterior descending branch of the left coronary artery, RCX: circumflex branch of the left coronary artery

Genotyping was successful in 2,865 patients for the 5352G/A (L235L) polymor phism of the caspase-1 gene, in 2,850 patients for the 7464C/G (A124G) poly morphism of the IL-1r gene, and in 2,761 patients for the 1858C/T (R620W) polymorphism of the PTPN22 gene. Genotyping for all three polymorphisms was successful in 2,676 patients. The results of the remaining patients are miss ing due to lack of DNA or inconclusive genotyping (vaguely positioned dots or genotyping errors).

The distributions of the genotypes are shown in table 2. The genotype distribu tions were consistent with the Hardy-Weinberg equilibrium ($p>0.05$).

Of the 3,104 patients, 304 (9.8%) patients underwent TVR during follow-up, 51 patients died (1.6%), and 22 (0.7%) suffered from MI.

By univariate analysis we observed a significant association between the caspase-1 polymorphism and TVR (p=0.001). TVR occurred more often in patients who were homozygous for the caspase-1 A-allele (19.1%) than in GA heterozygous patients (9.8%) or GG homozygotes (9.1%). The other two polymorphisms did not show a significant association with TVR.

Table 2. Univariate analysis of investigated polymorphisms in association with TVR and the distributions of the polymorphisms

Polymorphisms	Number of patients with and without TVR genotyped (N,%)	Best fitting genetic model	Number of patients with TVR for the different gaenotypes (N, %)	P-value*
Caspase-1 (5352G/A)				
GG	1,947 (68.0)	Recessive	178 (9.1)	**0.001**
GA	829 (28.9)		81 (9.8)	
AA	89 (3.1)		17 (19.1)	
IL1r (7464C/G)				
CC	2,483 (87.1)	Dominant	237 (9.5)	0.71
CG/GG	367 (12.9)		37 (10.1)	
PTPN22 (1858C/T)				
CC	2,249 (81.5)	Dominant	213 (9.5)	0.44
CT/TT	512 (18.5)		53 (10.4)	

**P value of association of particular polymorphism with TVR*

In a multivariable Cox regression analysis we included all three polymorphisms, and selected in a backward stepwise manner the polymorphisms that were independently associated with TVR risk. Polymorphisms were removed from the model when their p-value was >0.10. In this model patients with the caspase-1 AA-genotype still showed a significantly increased risk of TVR (p=0.004). Finally, we included in the multivariable regression model patient-related and intervention-related characteristics that were previously found to be related to TVR risk, such as diabetes, hypertension, stenting, residual stenosis>20%, current smoking and total occlusion. Furthermore, we included age and gender. Backward stepwise selection yielded the same polymorphism as found in the absence of clinical

factors, plus diabetes, hypertension, stenting and total occlusion (Table 3).

Table 3. Multivariable Cox regression of polymorphisms associated with TVR, including clinical factors

		95% CI		
	RR	Low	High	P-value
Diabetes	1.58	1.17	2.13	0.003
Hypertension	1.35	1.05	1.73	0.02
Stenting	0.65	0.50	0.84	0.001
Total occlusion	1.41	1.02	1.94	0.04
Caspase-1 5352G/A	2.23	1.32	3.76	0.003

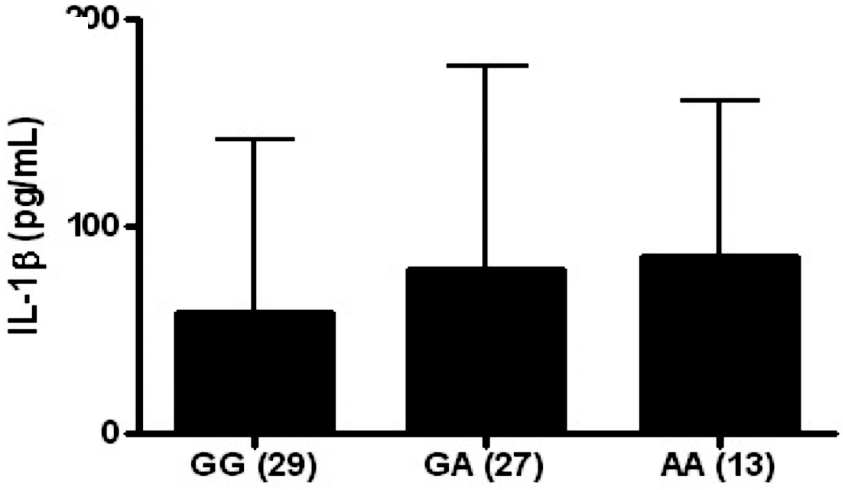
After subgroup analysis of the stented patient population, which consists of 2,133 genotyped patients, the caspase-1 5352AA genotype also increased the risk of TVR significantly (RR: 2.14, 95%CI: 1.16-3.93, p=0.02). Multivariable analysis on this subgroup population, corrected for the same clinical factors as mentioned above, showed a significant association for the 5352G/A caspase-1 polymorphism and TVR (p=0.03).

Additionally, six-month follow-up angiography was performed in a predefined subpopulation (478 patients). Since only 2 patients had the 5352AA genotype in the angiographic restenosis group, we combined the heterozygous patients with the patients homozygous for the variant allele. A significant association be tween patients with the 5352GA/AA genotype and angiographic restenosis was observed (OR: 2.26, 95%CI: 1.39-3.69, p=0.001). Angiographic restenosis rates were 16.4% for patients with the 5352GG genotype compared to 30.7% for pa tients with the 5352GA/AA genotype.

To further examine the role of the 5352G/A polymorphism, we measured mature IL 1β plasma levels with an ELISA test in lipopolysaccharide (LPS)-stimulated blood taken from a subgroup of 69 patients, who underwent their PCI in the Leiden University Medical Center. Since all stimulations performed showed a similar effect, we only present the data of the 1ng/μL LPS stimulation (Figure 1). Twenty-nine patients had the GG-genotype, 27 the GA genotype and 13 patients

were homozygous for the A-allele. Somewhat higher IL-1β levels were found in individuals with the 5352AA genotype (85.4 pg/mL (IQR: 125.7) for the AA geno type compared to 79.0 pg/mL (IQR: 119.9) for the GA genotype and 58.3 pg/mL (IQR: 118.5) for the GG genotype). However, differences between the groups were not statistically significant (p=0.28) in this small subset of patients. Since all patients included in this study have atherosclerotic lesions, inflammatory mark ers are possibly already activated, therefore we also examined the baseline levels of IL 1β, where also somewhat higher levels were found for the AA genotype of the caspase-1 gene (p=NS, data not shown).

Figure 1.



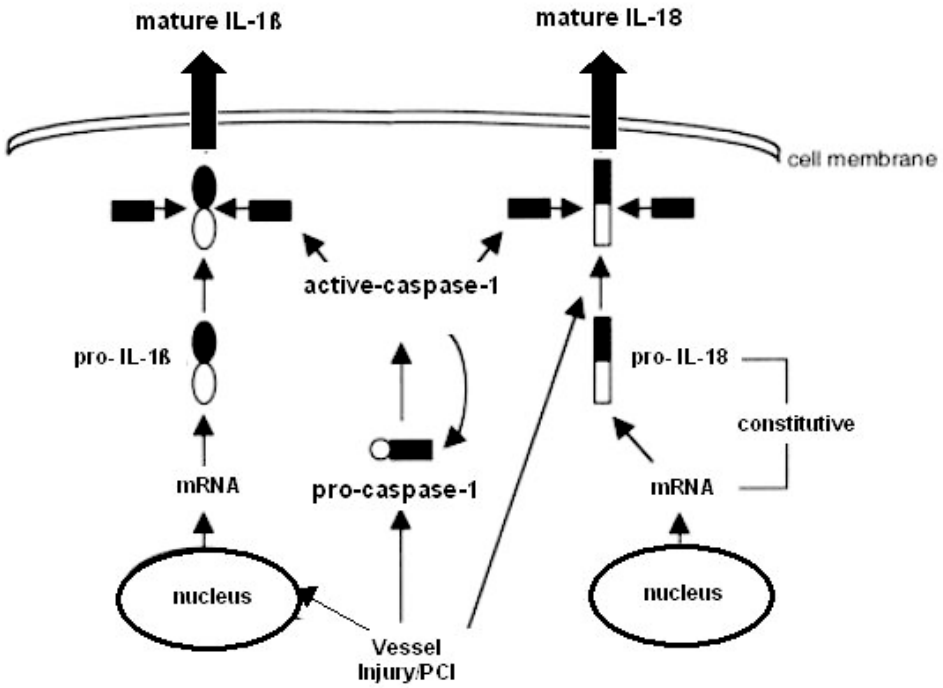
Plasma IL 1β levels in lipopolysaccharide 1ng/μL stimulated blood of 69 patients selected for having the GG, GA, AA genotype of the 5352G/A polymorphism of the caspase 1 gene p=0.28 . Indicated are median values and interquartile range.

Discussion

In this large prospective follow-up study, we examined three different polymor phisms in the genes encoding caspase-1, IL-1r and PTPN22. Our assumption that a relationship could exist between these genes and the development of re stenosis after PCI was based on observations suggesting a critical role of these genes in the process of inflammation and for caspase-1 also in apoptosis, which are known to play a pivotal role in the development of restenosis. Indeed, we found the 5352AA genotype of the caspase-1 gene to highly significantly increase the risk of developing restenosis after PCI, whereas no significant association for the polymorphisms in the other two genes could be demonstrated. Also an giographic analysis of a subgroup of patients showed an increased risk for devel oping restenosis for patients with the 5352GA/AA genotype.

Caspase-1, also known as IL1-β converting enzyme (ICE), is a member of a large family of intracellular cysteine proteases known as caspases. Caspase-1 subserves two dichotomous biologic roles. It induces cellular apoptosis through the cleav age of key intracellular structural and regulatory proteins and through the cata lytic activation of other caspase family members. More importantly, the predom inant role of caspase-1 in monocytic/ macrophagic cells is to process pro-IL-1β to yield active IL-1β and to cleave pro-IL-18 into active IL-18 (Figure 2). IL-1β is a cytokine, which plays a pivotal role in inflammatory cell activation and is known to inhibit the expression of neutrophil apoptosis.[19] Thus, caspase-1 may have di vergent effects on cell survival, dependent of the substrates to be processed.[8;20;21] IL-18 acts via an IL-18 receptor complex. IL-18 stimulates the inflammatory re sponse, however not directly; it acts together with IL-12 as a costimulant. IL-12 is released by a caspase-1-independent mechanism. IL-18 and IL-12 stimulate T cells and NK cells to release several lymphokines, TNFα, and Fas ligand (FasL). In turn, these cytokines stimulate macrophages to release TNFα, FasL, IL-8 and IL 1β, which stimulate inflammation.[21] In addition, caspase-1 is also required for the efficient expression of IL-1α, for reasons that remain unclear.[20] Because of the central role caspase-1 plays in the immune-cascade involving different in terleukins and TNFα and its role in apoptosis, caspase-1 qualifies as a candidate for influencing the development of restenosis.

Figure 2. The caspase-1 (interleukin-1β converting enzyme, ICE) pathway



Synthesis, caspase 1 processing, and secretion of IL β and IL 18. A human monocyte is shown. After cell stimulation, mRNA for pro IL 1β is induced and enters the cytosol. Pro caspase 1 is cleaved into active members of the caspase family including caspase 1 itself. Pro IL 1β is found diffusely in the cytosol and is cleaved by active caspase 1 into mature IL 1β, which is secreted from the cell. Pro IL 18 is expressed constitutively, as is the IL 18 mRNA. Af ter stimulation of the monocyte, pro IL 18 is cleaved by activated caspase 1 and released. Adapted from Siegmund et al.[21]

Unlike the 5352G/A polymorphism of caspase-1, polymorphisms in two other genes, i.e. the 7464C/G polymorphism of the IL-1r gene and the 1858C/T poly morphism of the PTPN22 gene, showed no a relationship with the occurrence of TVR in our patient-population. These polymorphisms have, to our knowledge, not been examined in relation to restenosis before.

The functional effect of the caspase-1 5352G/A polymorphism was not known

thus far. To predict the development of restenosis in an individual patient, plasma determinations will not have much additive value, since basal (pre-PCI) plasma measurements of the gene product may not likely reflect the genetically deter mined differences in reaction to a trauma such as PCI. To further increase our understanding of the effect of the caspase-1 polymorphism in the development of restenosis, we examined its possible functional effect under stress conditions. We found somewhat higher mature plasma IL-1β levels for patients with the AA genotype, however these results have to be interpreted with care because they were not significant in this small group. Therefore, further research is needed to find out whether this caspase-1 polymorphism influences the process of resteno sis directly or via other cytokines, like TNFα.

The caspase-1 polymorphism associated with TVR in our study may not be func tional itself but be in linkage disequilibrium with other polymorphisms in the gene or with other nearby genes that are actually responsible for the develop ment of this condition. Caspase-1 is
located in a cluster of genes on chromosome 11. The caspase-4, caspase-5, cas pase-1 dominant-negative inhibitor pseudo-ICE (COP1), inhibitory caspase re cruitment domain (INCA) and the ICEBERG genes are located in a 200 kb span encompassing caspase-1. No or weak LD was seen between the caspase-1 gene with the caspase-4, caspase-5, INCA and ICEBERG genes using Hapmap (www.hapmap.org) and Haploview, making the individual effect of caspase-1 more likely. However, strong linkage with COP1 was seen and therefore we can not exclude that the functional variant is located on the COP1 gene and only in LD with a marker on the caspase-1 gene. COP1 may affect restenosis via critical negative regulation of p53.[22] Furthermore, the possibility still remains that the effect of this polymorphism is detected due to LD with other polymorphism(s) within the caspase-1 gene.

In the era of drug eluting stents (DES) the question arises: do we need gene re search, since the rate of restenosis has reduced significantly with this therapy? DES have been shown to reduce the incidence of restenosis after primary angio plasty by targeting the proliferating vascular smooth muscle cells. However, late thrombosis and increased severity of intimal disease, intrinsic drug resistance and late development of aneurysm are limitations of this treatment.[3] Further more, they are more expensive and long-term experience with DES in coronary arteries is awaited. Until DES are sufficiently improved and broadly used, the conventional stents will still be used. Therefore the results of our study will be of interest for clinical practice for the coming years. Furthermore, the knowledge

on restenosis that is generated by this and similar studies will help in designing the drugs that will be used for coating the DES.[3] Several studies have investi gated the role of caspase-1 inhibitors in other inflammatory diseases. ICE inhibi tors were shown to prevent IL-1β maturation and therefore may serve as a new target protein for drug-eluting stents.[21]

The polymorphisms we examined in this current study have circumstantial evi dence from theoretical points of view/literature to be involved in the develop ment of restenosis. Furthermore, our study has enough power to detect a relative risk of 1.25 or more. The genotype we demonstrated to be significantly associat ed with restenosis, gave a predictive value in carriers over and above established risk factors and there was no significant evidence for heterogeneity of risk effect. Therefore, we believe that the polymorphisms we examined in the present study meet the criteria for a genetic variant to be included in clinical risk management of patients with CVD, which have recently been put forward by Humphries et al., satisfactorily.[23]

Study limitations

The caspase-1 showed strong linkage with COP1 and therefore we cannot ex clude that the functional variant is located on the COP1 gene and only in LD with a marker on the caspase-1 gene. Secondly, the subgroup of patients that was studied in a whole blood stimulation protocol was probably too small to detect significant differences in plasma IL-1β levels between the three caspase-1 geno types. Furthermore, the LPS stimulation may be insufficient to mimic in vivo events post-PCI. Finally, as our study was conducted in a sample of Caucasian patients, extrapolation of the data to other ethnic groups should be done with great caution.

Conclusions

The present study shows that caspase-1 (IL-1β converting enzyme), an important factor in the inflammatory response and apoptosis is a risk factor for the devel opment of restenosis. Patients with the 5352AA genotype were at increased risk for development of restenosis. This finding supports the hypothesis that genetic programming of the inflammatory response may be relevant to the pathogenesis of restenosis after PCI. Furthermore, when confirmed and mechanistically ex plained by independent studies, screening patients for this genotype may con tribute to a better risk stratification of patients at increased risk for restenosis and may improve individual treatment, for instance by using novel drug-eluting stents.

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