New insights in mechanism, diagnosis and treatment of myocardial infarction

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CHAPTER 8

GENETIC DETERMINANTS OF ADVERSE OUTCOME (RESTENOSIS, MALAPPOSITION AND THROMBOSIS) AFTER STENT IMPLANTATION

SUMMARY

Despite its unequivocal superiority compared with balloon angioplasty, coronary stenting did not abolish the restenosis problem and even brought along a completely new type of pathology. Bare-metal stents still associate with an approximate 20-30% in-stent restenosis rate and the need for repeat revascularization. Drug-eluting stents (which unfortunately did not completely prevent restenosis either) sometimes determine late-acquired stent malapposition in a significant number of patients. This is followed occasionally by a very serious event – stent thrombosis. Patient comorbidities, stent design, procedural characteristics and antiplatelet therapy influence the risk of poststenting complications. Research in the recent years has also revealed that individual genetic profile plays an important role in adverse outcome after stent implantation. This manuscript reviews the evidence of genetic variations associated with stent restenosis, late-acquired stent malapposition and stent thrombosis.

Keywords: coronary disease, stent, restenosis, malapposition, thrombosis, genes

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The era of percutaneous coronary intervention (PCI) began with the first balloon angioplasty performed by Andreas Gruentzig in 1977 [1]. Although this technique provided impressive immediate results, mid and long term follow up was characterized by high restenosis rates and need for repeat revascularization [1,2]. Evolving our techniques, bare-metal prosthetic devices (stents) were designed to act as a barrier against intima growth and recoil, assuring long-time patency of the coronary vessel. In 1986 Sigwart and Puel implanted the first coronary stent in a human patient [3]. Superior to balloon angioplasty alone (32-42% restenosis rate), bare-metal stent (BMS) implantation remains however vulnerable to restenosis (22-32% of cases) [4,5,6] and often requires re-intervention. Drug-eluting stents (DES) were conceived as an answer to this problem. They, for the majority, consist of a metalic platform covered with a combination of polymer and cellular proliferation inhibitor. The antiproliferative agent is gradually released in the arterial wall at the site of stent deployment preventing restenosis. The first successful DES trials were with sirolimus stents and led to their approval for use in 2002 in Europe and 2003 in USA [7,8]. Currently, other DES based upon paclitaxel, everolimus, zotarolimus, biolimus and tacrolimus are available. DES have successfully achieved their task of preventing restenosis but the experience of the last years revealed an increased incidence of stent malapposition and stent thrombosis associated with their use [9]. The aim of this article is to briefly present incidence and mechanisms of 1) stent restenosis, 2) stent malapposition and 3) stent thrombosis and to focus on potential genetic factors related to these complications. The majority of available data is retrieved from candidate gene approach studies, limiting thus the results to specific pre-targeted pathophysiologic sequences. Further novel pharmacogenomic approaches such as GWAS (genome wide association studies) may be able to identify new genetic factors for a better prediction of outcome after coronary stent deployment.

IN-STENT RESTENOSIS

In-stent restenosis (ISR) is defined angiographically when neo-formation tissue represents more than 50% of the lumen diameter at the site of the stented vessel (Figure 1).

The clinical confirmation of ISR is the recurrence of angina pectoris, which further requires intervention: TLR (target lesion revascularization) or TVR (target vessel revascularization). Although the severity of angiographic stenosis correlates with the need for TLR, half of the patients with angiographically confirmed ISR do not manifest clinical complains [6,10]. For this reason authors generally prefer to conduct their research in relation to angiographically documented ISR when an insight in the mechanism of restenosis is aimed, while studies comparing different stents are in relation to clinically-driven TLR or TVR.

ISR is the result of in-stent cellular proliferation and migration along with extracellular matrix accumulation [11]. Classic predictors of angiographic ISR (both in

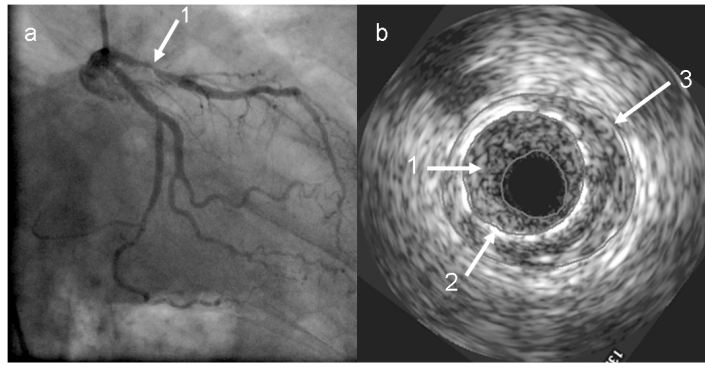

Figure 1. In-stent restenosis. a) Angiographically documented in-stent restenosis; b) IVUS documented in-stent restenosis. 1 – neointima; 2 – stent contour; 3 – vessel contour.

BMS and DES) include diabetes, renal failure, lesion length, reference vessel diameter and post-intervention lumen area [12,13]. Inflammation plays a pivotal role in ISR and it is triggered by the vascular injury during the stent deployment and by the presence of stent struts within the vessel wall [14,15]. Together with inflammation, major contributors are smooth muscle cell migration and proliferation but the process of restenosis involves many different cell-types, among which platelets and endothelial cells, and is also characterized by thrombus formation and to a lesser extent by matrix remodelling.

GENETIC FACTORS RELATED TO IN-STENT RESTENOSIS

Genetic variations in thrombus formation

In principle, any vascular intervention initiates the formation of a thrombus. Initial studies have shown associations of only a few polymorphisms in the hemostatic system with the risk for adverse events following a PCI. These early reports showed significant associations of the PLA1/A2 polymorphism with acute stent-thrombosis and coronary restenosis [16,17]. However, other studies in this field could not confirm these associations [18,19]. On grounds of the hypothesis that carriers of the PLA2 allele have a more intense binding of fibrinogen and vitronectin and thus a higher risk of platelet-rich white thrombus formation, the PLA2 allele can be expected to lead to an increased risk for acute stent thrombosis. However, as platelet inhibition by IIb/IIIa and P2Y12 antagonists does reduce acute stent thrombosis, but not in-stent restenosis rates [20], thrombus formation is probably not a main player in the development of restenosis. This hypothesis is further confirmed by findings showing that especially the strong pro-thrombotic genetic risk factors for venous thrombosis do not increase the risk for restenosis [21]. Moreover, results from the GENDER study [21] have shown

that the Factor V Leiden polymorphism (a well-known prothrombotic risk factor) was even found to reduce the risk for restenosis after PCI. A total of 3104 consecutive patients with stable angina pectoris or non-STEMI, of whom 2309 (74.4%) received stents, were included [21]. The factor V (1691 G>A or factor V Leiden) amino acid substitution was associated with a decreased risk of TVR (HR=0.41, 95%CI 0.19–0.86). The Factor V allele, which is known to lead to increased activation of protein C, might therefore influence restenosis risk by mechanisms not involved in coagulation, but in processes that have a more prominent role in neointimal growth, such as inflammation. Even though in another study of the same patient sample, associations were found between P2Y12 receptor haplotypes and restenosis [22], fewer and smaller effects were present in the stented subgroup. The decrease of the effects in this group could be due to inhibition of this receptor by clopidogrel (although several studies [23-26] failed to demonstrate a functional role of the P2Y12 receptor polymorphism in patients receiving dual antiplatelet therapy). Therefore, the genetic variation in this receptor, and also in many other genes with a role in the hemostatic system, may have been more important at a time in which not every patient was receiving a stent and concomitant platelet inhibition.

The 4G/4G genotype of the PAI-1 4G/5G polymorphism determines higher PAI-1 levels in plasma [27-29] and tissue [30-32]. The PAI-1 4G allele was associated with an increased risk of restenosis after PCI in the GENDER study [21]. When compared to 5G/5G homozygotes, heterozygous patients were at higher risk for clinically-driven TVR (HR=1.46, 95%CI 1.05–2.03), whereas patients with the 4G/4G genotype had an even further increased risk (HR=1.69, 95%CI 1.19 – 2.41). Although one smaller study could not confirm this association [33], many reports found a positive correlation between post-PCI PAI-1 levels or activity and restenosis [34,35]. Nevertheless, PAI-1 has a diverse role in several processes involved in restenosis, also in inflammation and proliferation [36]. Even if the 4G allele would increase the risk for restenosis, this could be mediated by a mechansism not related to fibrinolysis inhibition. Taking these findings together, we suggest that coagulation is not a main determinant of the long-term process that leads to restenosis.

Genetic variations in inflammatory factors

Early studies investigating the role of genetics in restenosis showed associations between variants in genes encoding cytokines [37] and selectins [38] – important mediators of inflammation – and suggested a role for inflammation in restenosis. One of these studies was performed by Kastrati et al.[37], and included 1850 consecutive stented patients. They demonstrated a protective effect of allele 2 of a polymorphism in exon 2 of the gene encoding the IL-1 receptor antagonist (IL-1ra), an anti-inflammatory interleukin, on both angiographic and clinical restenosis (OR=0.78, 95%CI 0.63-0.97 and OR=0.73, 95%CI 0.58-0.92, respectively). Monraats et al. have further established the important role of inflammatory genes in the development of restenosis. In the GENDER study, the rare alleles of the -260 C/T polymorphism in the

CD14 gene, the 117 Ile/Thr polymorphism in the colony stimulating factor 2 gene (also known as granulocyte-macrophage colony stimulating factor, GM-CSF) and the -1328 G/A polymorphism in the eotaxin gene were associated with decreased risk of TVR [39]. Eotaxin is a chemokine which selectively recruits eosinophils and was previously reported to be elevated in plasma of patients with advanced atherosclerosis. After coronary interventions, eotaxin levels increase and remain high for at least 24 hours but no longer than 3 month [40].

Furthermore, the variant alleles of two promoter polymorphisms in the Tumor Necrosis Factor alpha (TNF-α) gene have been shown to protect against the development of restenosis [41]. Stented patients with the -238A/A genotype (HR=0.44, 95%CI 0.23-0.83) and patients with the -1031C/C genotype (HR=0.72, 95%CI 0.52-1.00) needed TVR less frequently. Several other inflammatory genes were shown to be involved in the process of restenosis in this cohort, among which interleukin 10 and caspase-1 (IL-1β converting enzyme) [42,43]. All these findings support the hypothesis that restenosis is largely (albeit not solely) determined by inflammation.

Genes involved in smooth muscle cell proliferation

Stents specifically aiming to inhibit inflammation (dexamethasone eluting stents) were not proven as effective as stents inhibiting both inflammation and cell proliferation [44]. Despite the fact that restenosis is mainly determined by proliferation and migration of vacular smooth muscle cells (VSMCs), relatively few studies investigated genes involved in proliferation, such as cell-cycle regulatory genes. A recent important finding in this field by Van Tiel et al.[45] was an association between the -838 G/A polymorphism in the cyclin-dependent kinase inhibitor p27(kip1) (a key regulator of SMC proliferation) with ISR. Three polymorphisms concerning the p27(kip1) gene (-838C>A; -79C>T; +326G>T) were determined in a cohort of 715 patients undergoing coronary angioplasty and stent placement. Patients with the p27(kip1) -838AA genotype had a decreased risk of ISR (HR=0.28, 95%CI 0.10-0.77). This finding was replicated in another cohort study of 2309 patients (HR= 0.61, 95%CI 0.40-0.93). The -838 A allele corresponded to enhanced promoter activity which in turn may explain decreased SMC proliferation.

Genetic variations in matrix metalloproteinases

Matrix metalloproteinases (MMPs) are Zn^{2+} -requiring proteases capable of degrading a variety of extracellular matrix components. Due to their significance in vascular remodeling, MMPs are suspected to play an (important) role in the pathogenesis of atherosclerosis and restenosis [46]. Especially MMP2, MMP3 and MMP9 are potential players in the process of restenosis after PCI. MMP2 and MMP9 (the gelatinases) are produced by vascular VSMCs and degrade basement membrane components and other matrix proteins to allow migration and proliferation of vascular smooth muscle cells (VSMCs) [47]. They are upregulated and activated in VSMCs during intima formation in many different animal models for restenosis involving balloon

angioplasty [47]. An increase in MMP2 levels and activity was demonstrated in human coronary sinus blood samples 4 and 24 hours after elective coronary angioplasty [48]. This small study, in which only 21 of 47 patients were stented, also showed an association between MMP2 levels and restenosis. MMP3 (stromelysin-1) expression has been found to be related to plaque-instability in pathological studies [49]. MMP3 reduces the matrix content of the vascular wall and is therefore expected to protect against restenosis [49]. Functional studies have shown that the MMP3 -1612 5A/6A promoter polymorphism is associated with altered MMP3 expression. Carriers of the 6A/6A genotype were found to have a reduced MMP3 expression [50-53] and were at increased risk of developing restenosis in a subset of the REGRESS study, in which stents were not yet frequently used [54], and in two other studies with luminal narrowing after plain balloon angioplasty [55,56]. However, an association between the MMP3 5A/6A polymorphism could not be confirmed in a study which included 217 stented patients. Unpublished results from the GENDER study indeed show no association between this polymorphism and clinical restenosis in stented patients. Therefore, even though matrix formation is an important process in the development of restenosis, variations in genes involved in matrix remodeling were infrequently investigated or studies yielding negative results and were not published.

STENT MALAPPOSITION

Stent malapposition (SM), commonly detected by intravascular ultrasonography (IVUS), represents a separation of the stent struts from the intimal surface of the arterial wall (in the absence of a side branch) with evidence of blood speckles behind the struts [57] (Figure 2a).

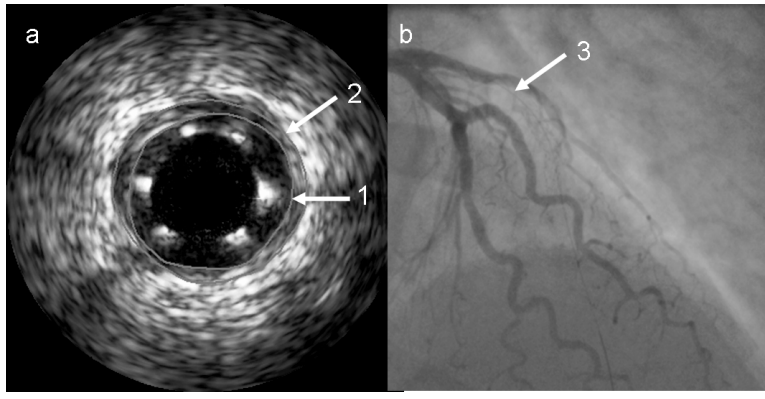

Figure 2. Stent malapposition and thrombosis. a) Intravascular ultrasound documented stent malapposition; b) angiographically documented stent thrombosis. 1 – lumen contour behind stent struts; 2 – vessel contour; 3 – stent thrombosis.

SM may be acute (present immediately after implantation), persistent (present both immediately after implantation and at follow-up) or late-acquired (present only at follow-up). Acute and persistent SM are mainly procedure-driven while late-acquired stent malapposition (LASM) is a consequence of positive remodelling of the vessel wall and and/or of plaque volume decrease behind the stent (including clot lysis or plaque regression) [58-62]. The main repercussion of late SM (persistent or acquired) is stent thrombosis (ST) [9]. Independent predictors of LASM include lesion length, unstable angina, absence of diabetes and primary stenting in acute MI [63,59]. The risk of LASM in patients with DES is approximately 4 times higher compared to those with BMS [9]. This is due to the fact that in BMS, hypersensitivity to the metallic stent is mostly associated with restenosis, whereas in DES, the hypersensitivity to the metallic stent, the polymer or to the drug is associated with positive remodelling and excessive inflammation in the vessel wall [64].

8 GENETIC FACTORS RELATED TO STENT MALAPPOSITION

We have previously investigated 7 polymorphisms (involved in inflammatory processes and related to restenosis) on the risk of LASM in SES patients [65]. In total, 104 STEMI patients from the MISSION! intervention study [62] were genotyped for the caspase-1 5352 G/A, eotaxin 1382 A/G, CD14 260 A/G , colony stimulating factor 2 1943 C/T, IL10 -1117 C/T , IL10 4251 C/T and the TNF-α 1211 C/T polymorphisms. LASM occurred in 26/104 (25%) of patients. We found a significantly higher risk for LASM in patients carrying the caspase-1 (CASP1) 5352 A allele (RR= 2.32, 95% CI 1.22-4.42). In addition, mean neointimal growth was significantly lower in patients carrying this LASM risk allele (1.6 vs 4.1%, p=0.014). The other 6 polymorphisms related to inflammation were not significantly related to the risk of LASM. Given the limited number of patients included in the study, similar reports are needed to confirm our findings. Moreover, a direct relation between the CASP1 5352 A allele and the risk of ST was not investigated. To our knowledge, no other studies yet scrutinized the role of genetic variations in LASM.

STENT THROMBOSIS

Stent thrombosis (ST) (Figure 2b) is a complication which occurs in 0.8-2% of patients undergoing PCI and is associated with large MI and death [66]. ST is categorized into "acute" ST (within 24 hours from stent implantation), "subacute" ST (within 1 – 30 days from stent implantation), "late" ST (within 30 days – 1 year) and "very late" ST (> 1 year after stent implantation). Subacute and acute ST are classically related to procedure parameters such as stent underdeployment (acute SM) [67,68] or procedure related complications such as coronary dissections [69,70]. In contrast, (very) late ST appears to be an active phenomenon associated with late SM

(persistent or acquired) [9,71], stent type [9] duration of dual anti-platelet therapy [66] and inflammation [58]. Gene variations in the platelet aggregation pathway, responsiveness to clopidogrel or presence of inherited thrombophilic disorders were associated with both acute and late ST.

GENETIC FACTORS RELATED TO STENT THROMBOSIS

Platelet receptor gene polymorphism

Platelet aggregation involves the binding of fibrinogen to the glycoprotein (GP) IIb/IIIa receptor on the platelet surface. One polymorphism of the GP IIIa gene (PLA1/A2 or HPA – 1a/1b) has been related to the inherited risk of coronary thrombosis [72]. Of importance, the same polymorphism had no influence on the degree of myocardial salvage achieved in 133 acute MI patients undergoing coronary stenting and abciximab administration [73]. The PLA2 polymorphism is a substitution of cytosine for thymidine at position 1565 in exon 2. Walter et al. [74] investigated the association of PLA2 allele with acute and subacute stent thrombosis in 318 consecutive BMS patients stented for coronary dissection, acute occlusion or high residual restenosis after PTCA lesions in by-pass grafts, and restenotic lesions. They found that patients with the PLA2 allele had and increased risk of stent thrombosis compared with patients homozygous for PLA1 (OR=5.26, 95%CI 1.55-17.85). Kastrati et al. [75] confirmed these findings partially in their prospective study including 1759 patients with stable and unstable angina pectoris. No difference was seen at 30 days after stent placement in terms of ST or a composite end-point of death, MI or urgent revascularization between PLA1/A1 and PLA1/A2 carriers. However, the incidence of ST and the composite end-point were higher in the PLA2 homozygotes versus PLA1 homozygotes (8.7% vs. 1.7%, p=0.002 and 13.0% vs. 5.4%, p=0.06, respectively).

More recently, Sucker et al. [76] assessed the relevance of prothrombotic platelet receptor polymorphisms for the onset of coronary stent thrombosis in 316 patients. They compared the prevalence of GP Ibα, GP IIb, GP IIIa (including PLA1/A2) and GP Ia prothrombotic polymorphisms in patients with coronary stent thrombosis occurring in the first 6 month after stent implantation and healthy control subjects. Carriers of the above mentioned prothrombotic versions did not appear to be at any increased risk for stent thrombosis. Selection of patients (differences in number of elective and acute stent implantations) and the treatment of more complex coronary lesions in the latter study or the limited power might explain these discrepancies [76]. Angiolillo et al. [77] have investigated the differential platelet sensitivity between PLA1 homozygotes and PLA2 carriers in 38 patients undergoing coronary stent implantation and receiving a 300 mg clopidogrel loading dose. They have shown that PLA2 carriers have a lower inhibition of platelet reactivity following the standard clopidogrel loading dose, which might finally lead to stent thrombosis.

Genetic variations in response to clopidogrel

In current practice, patients undergoing PCI and stent deployment are given 300-600 mg clopidogrel as a loading dose followed by 1 year dual anti-platelet therapy (aspirin 80-325 mg and clopidogrel 75 mg daily) and continued with life-long aspirin intake.

A good responsiveness to clopidogrel is therefore crucial in order to prevent thrombotic events after stent deployment.

Clopidogrel is an inactive prodrug which requires a two-step oxidation by the hepatic cytocrome P450 (CYP) enzymes to transform into an active metabolite which further inhibits the ADP P2Y12 receptor producing the anti-aggregation effect. The genes encoding the CYP enzymes are polymorphic and several variants were related to a decreased catalytic activity and subsequent attenuated effect of the drug.

The CYP3A5 gene has a functional polymorphism which includes the expressor (*1) and non-expressor (*3) alleles [78,79]. Suh et al. [79] compared clinical outcome in 348 patients (with stable angina, unstable angina or non-STEMI) who had PCI with BMS implantation. Antiplatelet therapy consisted of aspirin (100-300 mg daily, prescribed indefinitely) and clopidogrel (75 mg daily after 300 mg loading dose) administered for at least 4 weeks after the procedure. Atherothrombotic events (a composite of cardiac death, MI and non-hemorrhagic stroke) occurred more frequently within 6 months after stent implantation among the patients with the non-expressor genotype than among those with the expressor genotype (14/193 vs. 3/155, p=0.023). Moreover, the CYP3A5 polymorphism was a predictor of athrothrombotic events in clopidogrel users.

These findings are interesting especially since a number of studies (which did not aim at clinical end-points) found no association between the CYP3A5 variants and clopidogrel response and/or residual platelet aggregation (RPA) [80-82] nor did a number of studies with clinical end-points [83,84].

Trenk et al. [85] investigated whether the CYP2C19 681G>A *2 polymorphism was associated with high (>14%) RPA on clopidogrel and whether high on-clopidogrel RPA affects clinical outcome after elective coronary stent placement. RPA was assessed in 797 consecutive patients after a 600 mg loading dose and after the first 75 mg maintenance dose of clopidogrel before discharge. Patients were followed-up for 1 year. Between the *2 carriers and *1/*1 carriers (wild-type) the authors found significant ($p<0.001$) differences in the proportion of patients with RPA>14%, both after loading (62.4% vs. 43.4%) and at pre-discharge (41.3% vs. 22.5%). RPA >14% at discharge was associated with a 3-fold increase (95%CI 1.4-6.8, p=0.004) in the 1-year incidence of death and myocardial infarction. However, authors could not show a direct relation between the CYP2C19*2 allele and clinical outcome.

This relation was demonstrated by Giusti el al. [86] in a subanalysis of the RECLOSE trial. The role of the CYP2C19*2 polymorphism in the occurrence of DES ST (definite or probable) or the composite end-point of ST (definite or probable) and cardiac mortality within 6-month follow-up was assessed in 772 patients undergoing PCI and receiving either sirolimus or paclitaxel DES. Patients with ACS

and STEMI were included as well as patients with left main disease, chronic total occlusions, bifurcation lesions or diffuse disease. All patients received aspirin (325 mg) and a loading dose of clopidogrel 600 mg before the procedure followed by a maintenance dose of clopidogrel 75 mg and aspirin 325 mg daily. Patients with ST or ST and cardiac mortality end-point had a higher prevalence of the *2 allele (54.1% vs. 31.3%; p=0.025 and 51.7% vs. 31.2%; p=0.020, respectively). At multivariate logistic regression analysis, the CYP2C19*2 allele was an independent risk factor for ST (OR=3.43, 95%CI 1.01-12.78, p=0.047) and ST and cardiac mortality (OR=2.7, 95%CI 1.00-8.42, p=0.049).

Mega et al. [83] reconfirmed these findings on long term assessment of patients from TRITON-TIMI 38 study. A number of 1389 patients treated with clopidogrel who underwent PCI and stenting were followed-up for 15 months. Patients were initially admitted with non-STEMI (71%) and STEMI (29%). They received a 300 mg clopidogrel loading dose, followed by 75 mg daily maintenance dose for up to 15 months. For the CYP2C19, the presence of at least one copy of the *2 allele was associated with a higher rate of composite death from cardiovascular causes, non-fatal MI, non-fatal stroke (HR=1.42, 95%CI 0.98-2.05) and of definite/probable ST (HR=3.33, 95%CI 1.28-8.62) than did non-carriers.

Sibbing et al. [87] assessed the role of the mutant *2 allele of the CYP2C19 polymorphism on the 30-day incidence of definite ST in 2485 consecutive patients undergoing coronary stent placement. There are a number of differences with regard to the previous study [83]: (1) STEMI patients were excluded, (2) the end-point was acute and subacute definite ST and (3) patients received 600 mg clopidogrel loading dose.DES were used in 25% and BMS in 75% of the patients. Of the patients studied, 73% were CYP2C19 wild-type homozygotes (*1/*1) and 27% carried at least one of the *2 allele. The cumulative 30-day incidence of ST was significantly higher in CYP2C19*2 allele carriers vs. wild-type homozygotes (1.5% vs. 0.4%, HR=3.81, 95%CI 1.45-10.02, P=0.006). The risk of ST was highest (2.1%) in patients carrying the CYP2C19 *2/*2 genotype (p=0.002).

Recently, Collet et al. [88] demonstrated the role of the CYP2C19*2 allele in 259 young patients (aged <45 years) who survived a first MI and received clopidogrel treatment for at least a month. The primary endpoint was a composite of death, MI, and urgent coronary revascularization occurring during exposure to clopidogrel. The secondary endpoint was angiography-documented stent thrombosis Median clopidogrel treatment duration was approximately one year. The primary endpoint occurred more frequently in carriers than in non-carriers (15 vs. 11 events; HR=3.69, 95%CI 1.69-8.05, P=0.0005), as did stent thrombosis (8 vs. 4 events; HR=6.02, 95%CI 1.81-20.04, P=0.0009). The effect of the CYP2C19*2 genetic variant persisted from 6 months after clopidogrel initiation up to the end of follow-up (HR=3.00, 95%CI 1.27-7.10, p=0.009). The CYP2C19*2 genetic variant appeared the only independent predictor of cardiovascular events (HR=4.04, 95%CI 1.81-9.02, P=0.0006).

In a study [84] of 2208 patients presenting with acute MI (among which 1535 underwent PCI), patients carrying any two CYP2C19 loss-of-function alleles (*2, *3, *4, or *5), had a higher rate of death from any cause, nonfatal stroke, or myocardial infarction during 1 year of follow-up than patients with none (21.5% vs. 13.3%; adjusted HR=1.98; 95%CI 1.10-3.58). Among the patients who underwent PCI during hospitalization, the rate of cardiovascular events among carriers of CYP2C19 loss-of-function alleles was 3.58 (95%CI 1.71-7.51).times higher than among those with none.

For the development of a risk score for better prediction of RPA, Geisler et al. [81] analyzed the CYP2C19*2 genotype and previously identified non-genetic risk factors (age >65 years, type 2 diabetes mellitus, decreased left ventricular function, renal failure and acute coronary syndrome). They demonstrated a significant correlation of the non-genetic factors (χ^2 = 5.32; P = 0.021) and CYP2C19*2 (χ^2= 21.31; P < 0.0001) with high RPA, and the highest association for the combination of both (χ^2= 25.85; P < 0.0001). This was the first study to show that prediction of clopidogrel responsiveness may substantially be improved by adding CYP2C19*2 genotype to non-genetic risk factors. The important influence of the CYP2C19*2 genotype over platelet function and cardiovascular outcomes was recently confirmed by Shuldiner et al. [89] in the first GWAS paper identifying CYP2C19 as a candidate gene. In the Pharmacogenomics of Antiplatelet Intervention (PAPI) Study, clopidogrel was administered for 7 days to 429 healthy individuals and the response was measured by ex vivo platelet aggregometry. A GWAS was performed followed by genotyping the loss-of-function cytochrome CYP2C19*2 variant. The relation between CYP2C19*2 genotype and platelet aggregation was replicated in 227 clopidogrel-treated patients undergoing PCI (P = 0.02). Patients with the CYP2C19*2 variant were more likely (20.9% vs 10.0%) to have a cardiovascular ischemic event or death during 1 year of follow-up (HR=2.42, 95%CI 1.18-4.99, P = 0.02).

Factor V Leiden mutation

Factor V Leiden is the most common inherited thrombophilic disorder, resulting from a single mutation (1691 G>A) in the factor V gene. Individual heterozygous for this mutation are at increased risk for venous thrombosis, and in homozygous the risk becomes extremely high. Although conceivable, there is only one case report to document a possible relation between a factor V Leiden heterozygous patient and stent thrombosis (simultaneous occlusion of two stents, one in left anterior descending artery and one in the right coronary artery at 4 days after implantation in a patient receiving standard dual anti-platelet therapy) [90]. Further larger studies are therefore needed before factor V Leiden may be linked to ST.

LIMITATIONS

Many studies have managed to identify genes and polymorphisms involved in the post-stenting outcome after scrutinizing various plausible pathophysiologic mechanisms. However, to predict an accurate scale of adverse effects, an interaction assessment between genetic, non genetic (traditional risk factors) as well as epigenetic factors is of extreme importance. This information remains momentarily scarce.

Also of importance, findings from certain studies cannot sometimes be confirmed by other studies. This is largely explained by variation in study settings and therefore the replication of findings in independent studies needs to be further emphasized.

The candidate gene approach used to date in the majority of investigations narrows the results to specific areas of interest.

CONCLUSION

In-stent restenosis and stent thrombosis remain important limitations of the current PCI practice. Besides the procedure-related risk factors and medication, solid evidence shows that patient's own response to stent implantation influences the outcome. Individual genetic response involves inflammation, cellular proliferation, platelet receptors and drug metabolism pathways. A better understanding of the stent pathology has lead to the identification of new important genes and genetic polymorphisms. They may help us better identify the vulnerable patients who need extraordinary therapeutic measures. Conversely, genetic-epidemiologic studies have identified genes which subsequently have revealed important pathophysiologic mechanisms.

FUTURE PERSPECTIVES

The speed by which new genes are being related to stent pathology is matched by the speed of new developments in stent technology and medication. Novel pharmacogenomic approaches (e.g., GWAS, 1000 genome project) may help to identify unknown genetic factors for a better prediction of outcome after stent implantation [91].

It is however difficult to predict whether screening for established polymorphisms will prove, in the future, a cost-effective method for improved stent type selection or medication in the daily routine.

The classic stents appear to be rapidly being replaced by new and complex body-polymer-drug constructs that address most, if not all, of the current problems. The new generation of stents may appear capable of modulating local inflammation, to permit a good re-endothelization, to prevent stent thrombosis, to reduce the duration of anti-platelet medication and, if necessary, even to degrade after local healing is achieved.

New drugs such as prasugrel, ticagrelor and cangrelor seem to effectively inhibit platelet aggregation with little or no interindividual response variability. The combination of lessons learned form genetic and pathophysiologic studies, the newly available resources (e.g., stents, antiplatelet drugs and imaging) and refined implantation techniques will definitely improve PCI performances and extend its use.

EXECUTIVE SUMMARY

Genetic variants associated with an increased or decreased risk of in-stent restenosis (ISR) and stent thrombosis (ST)

In-stent restenosis (ISR)

» Genetic variations in thrombus formation
  Associated with decreased risk:
    Factor V 1691G>A (factor V Leiden) amino acid substitution
  Associated with increased risk:
    4G allele of the PAI-1 4G/5G polymorphism

» Genetic variations in inflammatory factors
  Associated with decreased risk:
    *2 allele of the IL-1ra gene
    T /T genoype of the CD14-260 C/T polymorphism
    Thr allele of the CSF2-117 Ile/Thr polymorphism
    A allele of the CCL 11 (Eotaxin) 1328 G/A polymorphism
    A/A genotype of the TNF -238 G/A polymorphism
    C/C genotype of the TNF -1031 T/C polymorphism
  Associated with increased risk:
    A/A genotype of the IL-10 -2849 G/A polymorphism
    A/A genotype of the IL-10 -1082 G/A polymorphism
    G/G genotype of the IL-10 +4259 A/G polymorphism
    A/A genotype of the Caspase-1 5352 G/A polymorphism

» Genes involved in smooth muscle cell proliferation
  Associated with decreased risk:
    A/A genotype of the p27(kip1)-838G/A polymorphism

Stent thrombosis (ST)

» Platelet receptor gene polymorphism
  Associated with increased risk:
    PLA2 allele of the GP IIIa PLA1/A2

» Genetic variations in response to clopidogrel
  Associated with increased risk:
    *3 allele of the CYP3A5 gene (encodes hepatic cytocrome P450 CYP enzymes)
    *2 allele of the CYP2C19 gene (encodes hepatic cytocrome P450 CYP enzymes)

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