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Original scientific paper

P2RY12 Gene Polymorphisms and Effect of Clopidogrel on Platelet Aggregation

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A B S T R A C T

Objective of this study was to assess platelet response to clopidogrel and its association with certain single nucleotide polymorphisms (SNPs) of the P2RY12 gene. Several studies have shown that patients with poor in vitro response to clopidogrel have worse outcomes after coronary interventions. Pharmacological response to clopidogrel is mediated by the $P2Y_{12}$ platelet receptor, therefore, SNPs of the P2RY12 gene may account for some of the observed variability in the cardiovascular risk. Fifty patients with stable coronary heart disease, undergoing percutaneous coronary intervention were included in this study. Response to clopidogrel was analysed using light transmitted aggregometry before, and 5 days after the initation of therapy. SNPs analysed: c.–15+742C>T, c.–180+2739T>C and c.18C>T. A higher proportion of non-responders to clopidogrel were noted in carriers of 18C>T[T/T] (p=0.05), and lower prevalence in carriers of 742C>T[T/T] (p=0.05). Participants with 742C>T[T/T] had significantly higher change in aggregation compared to other 742C>T variants ([C/C]=20.5±21.9%; [C/T]=20.0±31.2%; [T/T]=48.6±21.3%; p=0.03). Those carrying 18C>T[T/T] had smaller change in aggregation (7.6±15.0%) compared to other variants, but the difference was not statistically significant (p=0.15). Analysis of variance showed 18C>T[T/T] was a statistically significant predictor of poor response to antiaggregation therapy, independent from other clinical and demographic variables. There was no relation between poor response to clopidogrel and any other genetic variant. Our results suggest that 18C>T SNP of the P2RY12 gene may be an independent predictor of pharmacological response to clopidogrel. Larger prospective studies are needed to confirm this link and assess its possible clinical consequences.

***Key words**: coronary heart disease, percutaneous coronary intervention, platelet function, $P2Y_{12}$ receptor, P2RY12 gene, antiplatelet therapy*

Introduction

Platelet activation and aggregation is involved in the progression of the atherosclerotic vascular disease by triggering acute adverse events such as acute coronary syndrome (ACS) and stroke[1]. The adenosine diphosphate (ADP) $P2Y_{12}$ receptor plays a critical role in platelet activation[2]. This is the rationale for the use of $P2Y_{12}$ receptor antagonist clopidogrel in treatment of ACS and secondary prevention of cardiovascular (CV) events. Clopidogrel has been associated with a significant reduction in the combined risk of ischaemic stroke, myocardial infarction, or vascular death in population of patients with recent ischaemic stroke, recent myocardial infarction or symptomatic peripheral arterial disease[3]. However, cardiovascular events may still occur in patients treated with percutaneous coronary intervention (PCI) despite the use of clopidogrel. Stent thrombosis develops in 0.5–2% of PCI treated patients, while death, myocardial infarction or stroke occur in 8.5% of them. Finally, 21.4% of patients require revascularisation 1 year after PCI[4,5]. Significant interindividual variability in response to clopidogrel (prevalence of non-responders [change in plate-

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let aggregation ≤10%] ranges from 6% to 58%) may explain lack of protection in some of the treated individuals[6]. This is supported by recent studies that showed an association between *in vitro* platelet reactivity following administration of clopidogrel and the risk of new CV events after PCI[7–9].

There are a number of mechanisms that are involved in modulation of the clopidogrel effect on the $P2Y_{12}$ receptor, including variability in absorption, blood concentration of its active metabolite, or drug-drug interactions[10]. Resistance to clopidogrel may be determined by interindividual differences in number or activity of $P2Y_{12}$ receptors, cellular capacity to release ADP, or platelet activation via alternative pathways[11]. These mechanisms may be associated with genetic factors[12].

Several studies have analysed single nucleotide polymorphisms (SNP) of P2RY12 gene in order to assess their association with clinical CV outcomes or with the effect of clopidogrel on platelet function. It has been reported that in certain populations 742C>T is predictive of the peripheral arterial disease and platelet aggregation. In addition, 2739T>C was associated with the risk of stent restenosis and with response to administration of clopidogrel and 18C>T may be predictive of the risk for neurological events and showed an association tendency of increase in ADP-induced platelet aggregation[13–18].

In this study we assessed the effect of clopidogrel on platelet aggregability and whether the platelet response to clopidogrel associates with certain SNPs of several P2RY12 gene loci in a group of individuals with angiographically proven coronary heart disease scheduled for PCI.

Material and Methods

Patients

Fifty patients with stable coronary heart disease were included in the study. All participants had previously undergone coronary angiography, were diagnosed with significant coronary artery obstruction (defined as coronary artery stenosis >50%) and were scheduled for PCI. In addition to angiographically confirmed stable coronary heart disease, participants must have been between 18 and 80 years old. Those who met one or more of the following criteria were excluded from the study: acute bleeding, acute coronary syndrome, stroke within last 3 months, prothrombin time (PT) >1.5, platelet count <100,000/mm^3, hematocrit <0.25, creatinine >177 mmol/dm^3, and/or continuous thienopyridine therapy before the entry visit. Patients were recruited between November of 2007 to March of 2009. The study was approved by the Institutional Ethics Review Board of the University Hospital »Sveti Duh« in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant.

Study design

After signing the informed consent patients were hospitalized, medical history was taken and physical exam was performed. Blood samples were obtained for hematological and biochemical tests, platelet aggregometry and genotyping. Patients were then administered the loading dose of clopidogrel (300 mg), followed by the maintenance dose (75 mg per day). PCI was performed the following day. All patients received 100 IU/kg of heparin intracoronary during the intervention folowed by 100 mg of aspirin daily. Five (5) days (±1 day) after administering loading dose of clopidogrel blood samples were obtained for post-treatment aggregometry measurement.

Aggregometry

Blood samples for aggregometry were collected in tubes containing 3.2% citrate. Light transmitted (turbidimetric) aggregometry (LTA) was performed in platelet-rich plasma (PRP) with a platelet count adjusted to (250 25) × 10^9/dm^3. Platelets were stimulated with 5 μM ADP. Aggregation was performed with Behring Coagulation Timer (BCT) (Dade Behring, Frankfurt, Germany). Aggregation was expressed as the maximal percentage change in light transmittance from baseline with platelet-poor plasma (PPP) as reference. Normal response to clopidogrel was defined as the difference (reduction) between baseline and post-treatment aggregometry measurement of more than 10%, while response ?10% was considered as indicating the lack of normal response („non-responders«), as previously described[19]. Standard deviation for repeated intraindividal measurements was previosly obtained and used as an estimate of variability for this method. It ranged from 3.6 to 7.7%, with day-to-day variation accounting for the majority of the variation, followed by operator variability[20].

Genotyping

Deoxyribonucleic acid (DNA) extraction from blood samples was performed using BIOROBOT EZ1 and EZ1 DNA blood extraction kit (Qiagen, USA) according to the manufacturer's instructions. Real-Time polymerase chain reaction (PCR) SNP analysis of three SNPs was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) and predeveloped TaqMan SNP genotyping assay reagents for three P2RY12 SNPs: rs2046934 (c.–15+742C>T), rs6787801 (c.–180+2739T>C) and rs6785930 (c.18C>T) (Applied Biosystems, USA). 742C>T SNP genotyping was used to tag the H1(742T) and H2 (742C) haplotypes[14]. Annotation of the selected SNPs is according to the nomenclature recommendations of the Human Genome Variation Society, using the cDNA sequence available under GenBank accession number NM_022788.3 as reference. PCR was carried out according to the manufacturer's protocol. Control samples covering three possible SNP genotypes for each SNP and no template control were run in parallel with tested samples in each experiment.

Other laboratory analyses

Routine laboratory analysis included complete blood count (analysis done on Sysmex XE 2100, Sysmex America, Inc., Mundelein, IL, USA); potassium, sodium, urea,

creatinine, serum glucose, aspartate transaminase, alanin transaminase, gamma-glutamyltransferase, uric acid, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides (testing performed using Olympus AU 400, Olympus, Mishima, Japan); prothrombine time, international normalized ratio, activated partial thromboplastin time and fibrinogen (measured on Siemens BCS-XP Siemens Healthcare Diagnostics, Deerfield, IL, USA). The coefficient of variation for fibrinogen was 2.9% and 7.2% within series for Control Plasma N and Control Plasma P, respectively. From day to day it was 1.6% and 3.4%.

Statistical analysis

The sample size for the study was defined based on the frequency of inidividual polimorphisms as described in the literature in a population similar to ours. Data reported by Fontana et al. indicate that at least 40 subjects from the Caucasian population should be sufficient to identify polymorphisms with frequency of 5% with a confidence interval (CI) of 95%[13]. Hardy-Weinberg equilibrium of genotype frequency was tested using Chi-square test. All SNP's genotype frequencies were in agreement with the Hardy-Weinberg equilibrium.

Descriptive statistics included means and standard deviation for continuous variables and counts and proportions (percentages) for categorical variables. Response to clopidogrel was calculated by subtracting baseline % aggregation from the measure of aggregation obtained at endpoint (after 5 days). ANOVA was used to test continuous variables and Fisher's test for categorial variables.

Analysis of variance was used to assess interaction between genetic and nongenetic factors to assess potential mechanisms responsible for differences in response to clopidogrel. The following categorical variables were included in the initial assessment: genotypes of each SNP (major variant homozygote, heterozygote, minor variant homozygote), gender, diabetes, smoking, hypertension, hyperlipidemia, previous myocardial infarction, previous stroke, previous PCI, previous CV event, statins, beta blockers, ACE inhibitors or Angiotensin receptor blockers (ARBs). The following continuous variables were also included: body mass index (BMI), high density lipoprotein cholesterol (HDL), fibrinogen and % aggregation after stimulation with epinephrine. The following variables were statistically significant with respect to the change in clopidogrel, as measured by change in platelet aggregation after 5-day tretament with clopidogrel: SNP 742C>T, SNP 18C>T, fibrinogen level, and previous PCI. There was a statistically significant correlation between the genotypes of SNPs 742C>T and 18C>T. For this reason and because of opposite effects of the homozygote genotypes of these SNPs (18T homozygote group was associated with poor response and 742C homozygote group had greater response to clopidogrel than the average), the final model excluded 742C>T to focus on 18C>T due to its potentially greater value in detecting individuals at higher risk of poor response and subsequent new CV events.

Statistical analysis was performed using the Statistical Analysis Systems, version 9.2, software (SAS Institute, Cary, North Carolina, USA).

Results

Patient disposition

Table 1 shows summary demographic and clinical characteristics of 50 individuals included in the study. The study population had a high prevalence of CV risk factors, including hypertension, hyperlipidemia, and used CV medications frequently, as expected in people with coronary heart disease (CHD). Approximately 26% of them had diabetes. All participants completed all procedures at baseline and the follow up visit, as planned.

TABLE 1
BASELINE PATIENT CHARACTERISTICS

	Mean±SD	Count	%
Age (years)	63.38±8.8		
Height (cm)	170.96±8.89		
Weight (kg)	83.52±14.05		
BMI (kg/m^2)	28.46±3.88		
Cholesterol (mmol/dm^3)	5.31±1.34		
LDL cholesterol (mmol/dm^3)	3.05±1.14		
HDL cholesterol (mmol/dm^3)	1.35±0.31		
Triglicerides (mmol/dm^3)	2.32±1.47		
Fibrinogen (g/dm^3)	4.49±1.08		
Prothrombine time (s)	104.4±14.67		
APTT (s)	27.12±4.02		
Male/Female		35/15	70/30
Obesity (Y/N)		42/8	84/16
Diabetes (Y/N)		13/37	26/74
Smoking (Y/N)		10/40	20/80
Hypertension (Y/N)		43/7	86/14
Hyperlipoproteinaemia (Y/N)		46/8	92/8
Statin therapy (Y/N)		45/5	90/10
Beta-blocker therapy (Y/N)		31/19	62/38
ACE inhibitor/ ARBs therapy (Y/N)		42/8	84/16

BMI = body mass index, LDL = low density lipoprotein, HDL = high density lipoprotein, APTT = activated partial thromboplastin time, ACE = angiotensin-converting enzyme, ARBs = angiotensin receptor blockers

Platelet aggregation and response to clopidogrel

Mean baseline (±SD) platelet aggregation for the overall group was 63.22±14.60%. One patient had low (<19%) aggregation before the initiation of clopidogrel. Figure 1A shows distribution of platelet aggregation data at baseline. Five days after the initiation of therapy with

clopidogrel mean (±SD) platelet aggregation was 38.26± 17.75%, with an average decrease of 23.18±23.32%. Figure 1B shows distribution of response to clopidogrel administration (after stimulation with 5 μM ADP) 5 days after the initiation of therapy. Fourteen individuals (28%) out of 50 who participated in the study met the criterion for resistance to clopidogrel (decrease in platelet aggregation ≤10%, mean change in aggregation±SD –3.71±13.60%).

Assessment of single nucleotide polymorphisms of the P2RY12 gene

Distribution of various SNPs for all three single loci of the P2RY12 gene analyzed (742C>T, 2739T>C, 18C>T) are shown in Table 2. For each category within an individual SNP a proportion of poor responders was analyzed. This data is presented in Table 3. In most of the subgroups suppression of platelet aggregation was between 20 and 30%. Only two out of 9 subgroups were significantly different from other subgroups within individual SNPs. In the minor variant homozygote (T/T) subgroup of 742C>T, which included 5 participants, there were no poor responders (p=0.05 for within the SNP comparison). In the minor variant homozygote (T/T) subgroup of 18C>T a high proportion of non-responders was observed (42.9%) (p=0.05 for within the SNP comparison). Three out of 7 participants from this subgroup had a suppression of initial platelet aggregability ≤10% (mean change in aggregation±SD was –5.67±13.61). These results indicated a possibility that the two forms of homozygote variations of the P2RY12 gene may be having the opposite effect on platelet aggregation. Figure 2 shows graphically the effect of clopidogrel therapy on platelet aggregation as measured by LTA for each genetic variant of each of the SNPs included in the study.

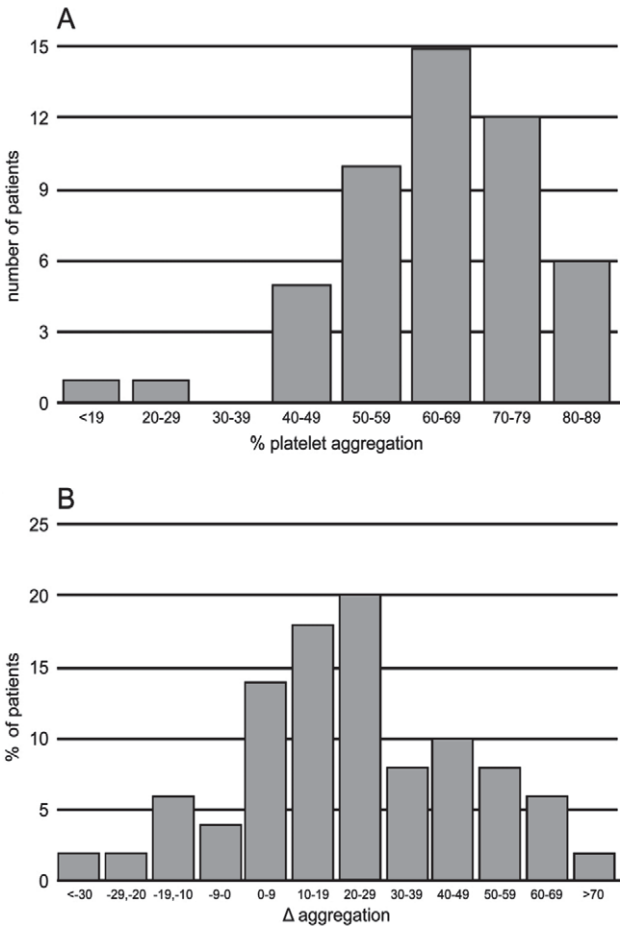

Fig. 1. A) Distribution of platelet aggregation data at baseline; B) Distribution of response to clopidogrel 5 days after the initiation of therapy.

TABLE 2
MEAN RESPONSE TO CLOPIDOGREL IN ADP STIMULATION TEST FIVE DAYS AFTER THE INITIATION OF TREATMENT.

	Major variant homozygote	Heterozygote	Minor variant homozygote	p
742C>T				
adjustright n	34	11	5	
% poor response	32.4	27.3	0	0.05
Mean Δ aggregation ± SD	20.5 ± 21.9	20.0 ± 31.2	48.6 ± 21.3	0.03
2739T>C				
n	14	20	16	
% poor response	28.6	30	25	0.07
Mean Δ aggregation ± SD	27.9 ± 30.6	18.0 ± 20.6	25.5 ± 19.1	0.43
18C>T				
n	24	19	7	
% poor response	25	26.3	42.9	0.05
Mean Δ aggregation ± SD	24.7 ± 24.7	26.9 ± 22.7	7.6 ± 15.0	0.15

Data presented for each subgroup defined based on the outcome of SNP assessment (major variant homozygote, heterozygote, minor variant homozygote).

TABLE 3
NUMBER OF POOR RESPONDERS TO CLOPIDOGREL IN EACH GENOTYPE CATEGORY OF THREE P2RY12 GENE SINGLE NUCLEOTIDE POLYMORPHISMS

SNP	742C>T				2739T>C				18C>T			
Genotype	C/C		C/T		T/T		T/T		T/C		C/C	
Patients (n)	34		11		5		14		20		16	
Response	>10%	≤10%	>10%	10%	>10%	≤10%	>10%	≤10%	>10%	≤10%	>10%	≤10%
Patients (n)	23	11	8	3	5	0	10	4	14	6	12	4

SNP	18C>T			
Genotype	C/C		T/C	T/T
Patients (n)	24		19	7
Response	>10%	≤10%	>10%	≤10%	>10%	≤10%
Patients (n)	18	6	14	5	4	3

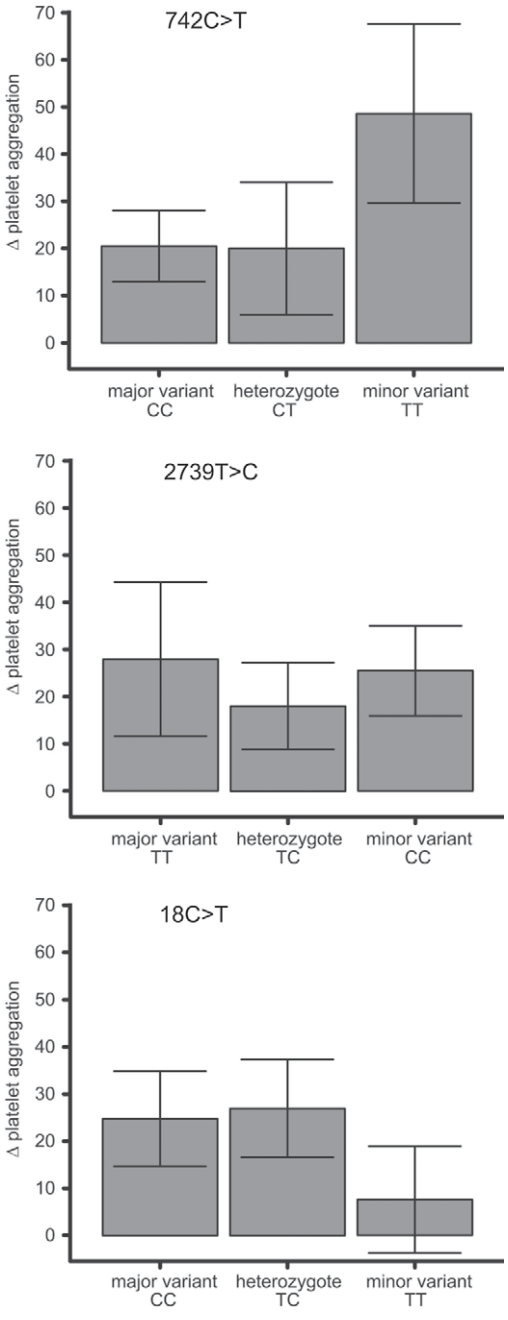

Fig. 2. Effect of clopidogrel therapy on platelet aggregation for 742C>T, 2739T>C and 18C>T SNPs of P2RY12 gene.

Relationship between platelet aggregation and SNPs of the P2RY12 gene (ANOVA)

Initial analyisis of variance model included the response to clopidogrel as dependent variable and genetic SNP variants, significant pre-existing conditions (abnormalities associated with increased CV risk, previous CV outcomes), and CV medications and laboratory measurements (HDL cholesterol, fibrinogen) as independent variables. In this model the strongest relationship was found between the response to clopidogrel and 742C>T, 18C>T, history of previous PCI, and concentration of fibrinogen. As described above, patients with minor homozygote variant 742C>T (T/T) displayed tendency towards greater than average response to clopidogrel and no resistance. Patients with minor homozygote variant of the 18C>T had a response that suggested a poorer response as compared to other variants of the same locus, as well, as other loci (Table 2). There was also an increased association between these minor homozygote variants of 742C>T and 18C>T, unabling definite separation of the effect of these two genetic variants on platelet activity. For these reasons and because of the previously reported data indicating lack of association between 742C>T and CV risk, the final analysis model excluded 742C>T polimorfisms. The final model showed that variants of 18C>T were the best predictor of inadequate platelet response to clopidogrel, independent from other variables that were included in this final analysis because of their statistically significant association with the response to clopidogrel in the primary assessment (fibrinogen and history of previous PCI).

Discussion

In this study we assesed possible relationship between the platelet response to clopidogrel administration and single nucleotide polymorphisms of its target receptor gene P2RY12 in patients with stable coronary artery disease undergoing elective coronary stenting. Increased prevalence of poor response to clopidogrel was associated with rs6785930 (18C>T) minor variant homozygous genotype (T/T). This relationship could not be explained by any other factor clinical or demographic, including those that showed strong association with the change in platelet aggregation during the therapy (fibrinogen, previous PCI). Since this was an exploratory study, with small number of participants included, these results should be

re-evaluated in a larger population using longitudinal follow up to include CV outcome data.

The study population encompassed a homogenous group of patients with stable coronary heart disease undergoing PCI. This population is commonly included in studies that assess genetic factors involved in the patophysiology of CV disease. These individuals are often treated with clopidogrel and understanding the effect of this drug on the platelet function and CV risk in this high-risk population is of clinical importance. As described in the Results section of this report, 28% of participants were non-responders to clopidogrel. This result was obtained using the light transmission aggregometry methodology, the gold standard method for platelet function testing[19]. To increase the accuracy of the platelet functional estimate, baseline and on-treatment measurements were obtained as is mandatory due to high inter-individual variability in platelet reactivity and in laboratory test responses[21,22]. The obtained proportion of non-responders is in the middle of the frequency range reported in the literature[19,23–25].

18C>T minor variant homozygous genotype (T/T) was associated with increased prevalence of individuals with poor response to clopidogrel in the study population, although no difference between the variants of this locus was observed in the magnitude of the response. This may be explained by small number of individuals in many of the subgroups and relatively high variability of the platelet aggregation. In the initial ANOVA models we found that additional factors were statistically significantly associated with the effect of clopidogrel on platelet aggregation, namely, concentration of fibrinogen, previous PCI and 742C>T polymorphism. Since 742C>T minor allele polymorphism had an opposite effect (increased response to clopidogrel) it was excluded from the final model which focused on variables predictive of poor response to clopidogrel. This final model indicated that variants of 18C>T were the best predictor of the platelet response to clopidogrel, independent from fibrinogen or history of previous PCI. In a study of P2RY12 SNP 18C>T by Fontana et al. no association between platelet aggregation and 18C>T polymorphism was observed[14]. In two studies of relationship between this SNP and clinical outcomes, Zee et al. reported no evidence of association with incident MI, ischemic stroke or DVT/PE[25], but Ziegler et al. reported that patients carriers of 18C>T had four-fold increased adjusted risk for neurological events despite therapy with clopidogrel[17]. Recently, Lee et al. reported that haplotype which contains 18C>T SNP showed an association tendency with 7% increase in ADP-induced platelet aggregation in healthy subjects when compared to individuals with the reference haplotype[18].

NCBI SNP database (dbSNP) currently has more than 200 listed SNPs in the region of P2RY12 gene which contains three exons, of which only exon 3 is coding. Even though 18C>T is positioned in exon 3, the change in nucleotides does not change the code for the amino-acid in that particular codone (asparagine). It is possible that 18C>T is a part of a haplotype that interferes with gene regulation within the P2RY12 coding region, which may have an effect on $P2Y_{12}$ receptor and consequently on platelet's response to clopidogrel.

The other two SNPs assessed in the study population (742C>T and 2739T>C) were already investigated in several populations. In our study patients who carry 742C>T minor variant polymorphism (T/T) (homozygotes) all responded well to clopidogrel therapy which is in agreement with the most of the recent reports, although most recent report from Lee et al. showed significant 8% increase in ADP-induced platelet aggregation in healthy male individuals carrying haplotype containing minor variant of 742C>T polymorphism compared with reference haplotype.[18] Rudež et al. initially reported that P2RY12 SNPs 2739T>C may be associated with altered ADP-mediated platelet aggregation and risk of arterial thrombosis.[27] In a subsequent report the same group suggested that 2739T>C predicts the risk of restenosis in patients treated with PCI and stenting. Results of a large cross-sectional study report that 2739T>C polymorphism is responsible for most of the haplotype effects on the action of clopidogrel on platelet aggregation. We found no significant difference in clopidogrel response regarding 2739T>C SNP in our population. The reason for this discrepancy may relate to different methodological approaches between the two studies. In their study (n=1031) Rudež et al. performed a single assessment of the platelet function after the initiation of treatment with clopidogrel (»on-treatment«) using LTA and VerifyNow P2Y12 assays. As mentioned before, intraindividual variability in platelet reactivity makes single-point-in-time tests in assessing the response to antiplatelet therapy less accurate as compared to multiple intraindividual measurements, which may be the reason for discrepancy from our results[15,16]. Most recent study from the same group reported that carriers of minor variant of 2739T>C polymorphism have increased response to cangrelor – novel irreversible P2Y12 inhibitor[28].

Further studies, involving more P2RY12 SNPs and evaluation of major and minor haplotypes in regards to standardized tests of the response to antiplatelet therapy are warranted to provide more accurate perspective on genetic determinants in patients with suboptimal response to agents that target $P2Y_{12}$ receptor.

There are several limitations of this study which include small study population and SNPs beeing analyzed. Also there was no clinical follow-up regarding clinical outcomes related to different platelet reactivity under clopidogrel treatment. At the time this study was conducted antiplatelet therapy guidelines advised a loading dose of clopidogrel of 300 mg so no subsequent comparison of 300 mg and 600 mg loading dose of clopidogrel could be done. In most previous similar studies diabetics versus non-diabetics had lower response to antiplatelet (clopidogrel) treatment. Our results have shown no statistical difference regarding response to clopidogrel between these two subgroups of patients.

Conclusion

Results of the study reported here suggest that 18C>T single nucleotide polymorphism of the target receptor gene P2RY12 is the best independent predictor of pharmacological response to clopidogrel, but larger prospective studies are needed to confirm the suggested link of 18C>T P2RY12 SNP with response to clopidogrel and possible clinical consequences that homozygotes of minor 18C>T allele might suffer.

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POLIMORFIZMI P2RY12 GENA I UČINAK KLOPIDOGRELA NA AGREGACIJU TROMBOCITA

SAŽETAK

Cilj ovog istraživanja bio je procjena odgovora trombocita na klopidogrel i njegove povezanosti s određenim polimorfizmima jedne baze (single nucleotide polymorphism – SNP) P2RY12 gena. Nekoliko je studija pokazalo kako bolesnici sa slabijim in vitro odgovorom na klopidogrel imaju lošije ishode nakon koronarnih intervencija. Farmakološki odgovor na klopidogrel posredovan je preko P2Y12 receptora, stoga bi SNP-ovi P2RY12 gena mogli biti odgovorni za dio primjećene varijabilnosti u kardiovaskularnom riziku. U ovo istraživanje bilo je uključeno pedeset bolesnika sa stabilnom koronarnom bolešću predviđenih za perkutanu koronarnu intervenciju. Odgovor na klopidogrel analiziran je metodom svjetlosne agregometrije prije te 5 dana nakon započete terapije klopidogrelom. Analizirani SNP-ovi: : c.-15+742C>T, c.-180+2739T>C and c.18C>T. Veća proporcija »loših odgovarača« na klopidogrel zabilježena je kod nositelja polimorfizma 18C>T[T/T] (p=0,05), a niža prevalencija kod nositelja 742C>T[T/T] (p=0,05). Ispitanici nosioci 742C>T[T/T] imali su značajnije veću promjenu u agregaciji trombocita u usporedbi s drugim varijantama 742C>T varijante

([C/C]=20,5±21,9%; [C/T]=20,0±31,2%; [T/T]=48,6±21,3%; p=0,03). Nosioci 18C>T[T/T] imali su manju promjenu u agregaciji trombocita (7,6±15,0%) u usporedbi s drugim varijantama, ali razlika nije bila statistički značajna (p=0,15). Analiza varijance pokazala je da je polimorfizam 18C>T[T/T] statistički značajan prediktor za slabi odgovor na antiagregacijsku terapiju, neovisan o drugim kliničkim i demografskim varijablama. Nismo otkrili drugu vezu između slabog odgovora na klopidogrel i neke druge istraživane genetičke varijante. Naši rezultati ukazuju da bi 18C>T SNP P2RY12 gena mogao biti nezavisni prediktor farmakološkog odgovora na klopidogrel. Potrebne su veće prospektivne studije kako bi se potvrdila ova povezanost te kako bi se procjenile njene moguće kliničke posljedice.