

Lack of association between α_{2B} -adrenergic receptor polymorphism and risk of restenosis following coronary angioplasty and stent implantation – preliminary report

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Abstract

Background: A genetic association/prospective follow-up study was conducted to investigate whether genetic variation of the α_{2B} -adrenergic receptor gene was associated with the risk of restenosis in 96 Greek coronary artery disease patients undergoing coronary angioplasty and stent implantation.

Methods: For comparison of genotype frequency, a control group of 83 asymptomatic individuals was also studied. The end-point of the current study was the incidence of restenosis at 7 months of clinical follow-up.

Results: The majority of patients (70/96) had the insertion/insertion genotype, fewer patients (23/96) had the insertion/deletion genotype and only 3/96 had the deletion/deletion genotype; overall the frequency distribution was not different from that of the control subjects. Restenosis occurred in 15 of the 96 patients.

Conclusions: In the population studied, α_{2B} -adrenergic receptor polymorphisms were not found to predispose patients to an increased incidence of restenosis. Nevertheless, these findings should be considered as preliminary, taking into account the small number of patients that were studied and the rarity of the deletion/deletion genotype.

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Keywords: α_{2B} -adrenergic receptor; coronary heart disease; polymorphisms; restenosis.

Introduction

Restenosis is a major concern following percutaneous transluminal coronary angioplasty (PTCA) and stent implantation in patients with coronary artery disease (CAD). Restenosis develops mainly within 3–6 months after PTCA and adversely affects the long-term efficacy of the procedure (1). Although the cellular and molecular basis of this process remains unknown, neointimal hyperplasia represents a primary mechanism and consists of smooth muscle cell (SMC) proliferation and migration that occurs subsequent to intimal denudation due to injury incurred during mechanical dilation of the lesion (2, 3). Factors involved in the proliferation of SMCs include endothelin-1, thrombin, lysophosphatidic acid and vascular endothelial growth factor (4). SMCs are well known to express multiple adrenergic receptor (AR) subtypes (5, 6) and their migration is controlled, among others, by activation of the α_2 -ARs (5).

ARs are essential components of the sympathetic neural circuitry regulating cardiovascular function (7–9). The α_2 -ARs are subdivided into α_{2A} -, α_{2B} - and α_{2C} -ARs, based on ligand binding and molecular biological criteria (10, 11). Polymorphisms are found in all α_2 -AR subtypes (12–14). In particular, polymorphisms occurring in the third cytoplasmic loop of the α_2 -AR modify the biochemical properties of its variants: a point mutation in α_{2A} -AR results in enhanced G protein coupling and a deletion (D) in the third cytoplasmic loop of the α_{2C} -AR results in reduced G protein coupling (15–17). An interesting polymorphism of α_{2B} -AR has recently been identified, involving an in-frame 9-bp deletion within a highly acidic region of the third intracellular loop of α_{2B} -AR (18). This results in the loss of three glutamic acid residues (Del301–303) that entails decreased agonist-promoted desensitization of the receptor (14). Recent clinical genetic studies in Finnish subjects showed that reduced receptor desensitization is potentially related to acute CAD (19) and impaired endothelial function (20), as well as to increased coronary and peripheral vasoconstriction upon acute systemic adrenaline infusion (21) and to increased sympathetic nervous system activity in young healthy individuals (22). In another study, middle-aged white men carrying the α_{2B} -AR DD genotype were found to have a significantly increased risk for sudden cardiac death and acute myocardial infarction (23).

Given the functional significance of α_{2B} -AR in SMCs and the association of α_{2B} -AR polymorphism with CAD, we conducted an association study of the (Del301–303) α_{2B} -AR gene polymorphism with the risk

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Table 1 Subject demographics and risk factors.

Clinical variable	Patients (n=96)	Controls (n=83)
Age, years	57.7 ± 10.1	35.2 ± 4.2
Male/female	81/15	41/32
Unstable angina	46	0
AMI	35	0
Systolic blood pressure (subjects with hypertension only), mm Hg	162.0 ± 9.8	157.7 ± 12.7
Diastolic blood pressure (subjects with hypertension only), mm Hg	98.5 ± 9.4	95.5 ± 8.1
Hypertension ^a	31	13
Smokers	54	40
Plasma total cholesterol, mmol/L	6.73 ± 1.10	5.57 ± 1.05
Plasma triglycerides, mmol/L	2.08 ± 0.90	1.83 ± 0.46
Hyperlipidemia ^b	86	44
Fasting plasma glucose (subjects with diabetes only), mmol/L	9.17 ± 1.94	8.89 ± 1.00
Diabetes ^c	18	12
Body mass index, kg/m ²	26.6 ± 4.7	29.4 ± 5.1
Obesity ^d	13	19
Positive family history ^e	28	12

Values are reported as mean ± SD or n. AMI, acute myocardial infarction; BP, blood pressure; CAD, coronary artery disease. ^a Systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg (31). ^b Total cholesterol > 5.17 mmol/L (24); ^c Fasting plasma glucose > 7 mmol/L (25). ^d Body mass index > 30 kg/m², ^e Family history of AMI, CAD or sudden cardiac death; data obtained during interviews.

of restenosis in 96 patients subjected to PTCA and stent implantation.

Subjects and methods

The possible connection of certain physical and biochemical variables with restenosis and α_{2B} -AR genotype was investigated. For comparison of genotype frequency, a control group of 83 asymptomatic individuals was also studied. The institutional Review Board approved the study, and informed consent was obtained from patients and control subjects for both the clinical procedures and the genotype determination prior to their inclusion into the study.

Patients

The present study comprised 96 consecutive patients with CAD, who underwent successful coronary angioplasty (PTCA) and stenting (Table 1). These were 81 men and 15 women (mean age ± SD, 57.7 ± 10.1 years, range 37–76 years) who presented with symptomatic CAD and were submitted to PTCA and stenting at the University Hospital of Patras. All patients were enrolled into the study between 2001 and 2003 and were followed up clinically for a mean of 7.0 ± 1.5 months after an initially successful procedure. All patients in the present study underwent coronary angiography and angioplasty procedures using standard techniques (26–30). Post-angioplasty, and for 1 month following this procedure, all the patients received aspirin (100–325 mg/day) and clopidogrel (75 mg/day) (31). All patients received at least one coronary bare metal stent. Assessment of stenosis was based on coronary angiography. Stenosis was defined as a narrowing of the luminal diameter of 75% or greater in one of the main coronary arteries or major branch, including the left anterior descending coronary artery (LAD), right coronary artery (RCA) or left circumflex coronary artery (LCX), except for the left main coronary artery, for which 50% narrowing of the lumen was considered significant. Assessment of restenosis was based on coronary angiography or thallium stress testing and was defined as recurrence of narrowing of the lumen diameter of the dilated vessel of 50% or more.

Control group

In addition to the patient group, a control group of 83 asymptomatic individuals, 41 men and 32 women (mean age 35.2 ± 4.2 years, range 42–73 years), of the same demographic ancestry as the studied patients, with no personal medical history of cardiovascular disease, but with several risk factors for cardiovascular disease (hypertension, diabetes, hyperlipidemia, obesity or smoking) were included in the study (Table 1). The control subjects underwent treadmill testing and the maximum chronotropic and inotropic responses to exercise were negative for CAD (no angina or ST-T depression or elevation was noted). The family history of control subjects was assessed through interview and was considered positive if acute myocardial infarction (AMI), CAD or sudden cardiac death before age 60 in first-degree kin (parents or siblings) was reported.

Genetic analysis

Blood from patients was collected into EDTA tubes at the time of coronary angioplasty. Genomic DNA was extracted from peripheral blood leukocytes using a Nucleospin Blood quick pure DNA isolation kit (Macherey-Nagel, Düren, Germany) and the polymorphic region of the α_{2B} -AR gene was amplified using the genotyping primer set, as previously described (18). Since there is no restriction enzyme to pinpoint this polymorphism, the allele variants (200 vs. 209 bp) were distinguished by their different mobility rates in high-density agarose gels (4%).

Statistical analysis

The genotype frequency distribution was compared for patients and control subjects. Hardy-Weinberg equilibrium was tested on genotype frequency according to Guo and Thompson (32).

Variables, such as hypertension (systolic blood pressure > 140 mm Hg and/or diastolic pressure > 90 mm Hg) (33), smoking, hypercholesterolemia (24), diabetes mellitus (25), obesity, gender and family history of previous AMI, CAD or sudden cardiac death, were tested as dichotomous confounding factors for restenosis and genotype distribution. Statistical analyses were carried out using Pearson's χ^2 (with

Yates' correction) and Fisher's exact tests. Computation analysis was performed using SPSS version 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). Quantitative results are expressed as mean \pm SD. Statistical significance was assumed for two-tailed values of $p < 0.05$.

Results

Distribution of the (Del301–303) α_{2B} -AR polymorphism

The allele frequencies of the polymorphisms complied well with Hardy-Weinberg equilibrium in both the patient and control groups. Most of the patients ($n=70$) and control subjects ($n=51$) were screened homozygous for the type *I* allele (Table 2): the frequency of the *I* allele was calculated to be 0.85 in patients and 0.79 in control subjects.

Restenosis occurred in 15 of the 96 patients. Analysis of restenosis with genotype and risk factors showed no association between variables (Table 3). Of the genotypic groups, restenosis was found in 11/70 for *I/I*, 3/23 for *I/D* and 1/3 for the *D/D* genotype. No association between gene polymorphisms and restenosis could be detected ($p=0.66$) in patients. Furthermore, in patients, restenosis was not associated with hypertension ($p=0.49$), smoking ($p=0.12$), hypercholesterolemia ($p=0.19$), diabetes ($p=0.89$), obesity ($p=0.70$), gender ($p=0.61$), or positive family history for AMI, CAD or sudden cardiac death ($p=0.70$).

High blood pressure was found in 23/70 patients of the *I/I* group, 6/23 of the *I/D* group and 2/3 of the *D/D* group. Patients with hyperlipidemia accounted for the majority of patients in all genotype groups (63/70 of the *I/I*, 20/23 of the *I/D* and 3/3 of the *D/D* group). None of the *D/D* genotype patients was diabetic, but 15/70 of the *I/I* and 3/23 of the *I/D* group were screened positive for diabetes. Table 4 shows that there was no significant association between α_{2B} -AR gene polymorphisms and the risk factors for hypertension ($p=0.36$), hypercholesterolemia ($p=0.77$) and diabetes ($p=0.47$).

Discussion

Risk factors for restenosis after PTCA include positive family history, male gender, the presence of unstable angina pectoris, diabetes and smoking (1, 34–48). The process of restenosis following PTCA is multifactorial and several mechanisms such as thrombus for-

mation, vascular wall remodeling and neointimal hyperplasia have been found to contribute to the pathophysiology of the process (35).

Previous studies have investigated the association between individual candidate gene polymorphisms, including angiotensin-converting enzyme (ACE), the AT1 receptor for angiotensin II and cholesteryl ester transfer protein (CETP), with restenosis in certain populations (36, 37). However, no prior association study has been performed with regard to the α_{2B} -AR polymorphism and restenosis and, to the best of our knowledge, this is the first analysis investigating the implication of this polymorphism for the risk of restenosis following PTCA and stenting. The present study was prompted by the fact that α_{2B} -ARs stimulate the migration of vascular SMCs (49), and thus they may participate in the process of neointimal hyperplasia, as well as by the association of the α_{2B} -AR deletion variant with cardiovascular events (19) and autonomic nervous system activity (22). In fact, polymorphisms in various other genes involved in vascular SMC proliferation and migration are considered to contribute to the development of restenosis following PTCA and stenting (50–52).

In contrast to a previous study (18), a very low allelic and genotypic frequency for the *D*-variant was observed in the present study, with most subjects, whether patients or control subjects, being homozygous for the *I/I* genotype ($n=70/96$). It is believed that the α_{2B} -AR *D*-variant is less common in certain ethnic groups than in others (53). However, a recent study in Greek subjects for the correlation of α_{2B} -AR polymorphism with morbid obesity (54), supported by our present results, indicated that there is a high variability in polymorphic genotype distributions within Caucasians. In the present study, we did not detect any statistically significant association of α_{2B} -AR polymorphism with restenosis, indicating that α_{2B} -AR polymorphism is not, on its own, a major risk factor for restenosis, at least in Greeks with CAD. Although other genetic polymorphisms or other pathological or environmental factors may mask the effects of the α_{2B} -AR polymorphism on the risk of restenosis, synergism between polymorphisms that contribute to the mechanisms of restenosis cannot be predicted at present. Of course, our negative findings should be considered as preliminary, taking into account the small number of patients that were studied and the rarity of the *D/D* genotype. Furthermore, there may be negative selection among the patients because of increased mortality (prior to inclusion in the study) associated with the genetic variation investigated, but at present we cannot avoid such limitations. Another limitation of the study is the fact that the follow-up period might have been short compared to the longer time needed for persons with unstable angina to develop restenosis (30).

In our study there was a low incidence of restenosis (15/96) at the 7-month follow up period. Most of our patients were men, smokers, non-diabetics and normotensive, with high cholesterol levels but no previous family history of AMI, CAD or sudden cardiac death.

Table 2 Genotypic distribution of α_{2B} -AR gene polymorphism in Greek subjects.

Subjects	Genotype, n			Total, n
	<i>I/I</i>	<i>I/D</i>	<i>D/D</i>	
Controls	51	28	4	83
Patients	70	23	3	96

Fisher's exact p (comparing patients and control subjects): 0.254.

Table 3 Statistical analysis of restenosis with genotype and possible risk factors in patients.

Variable		Restenosis, n			Pearson's χ^2 values		
		Yes	No	Total	χ^2	df	p
Genotype	I/I	11	59	70	0.83	2	0.66
	I/D	3	20	23			
	D/D	1	2	3			
Hypertension	Yes	6	25	31	0.48	1	0.49
	No	9	56	65			
Smoking	Yes	7	46	53	4.32	2	0.12
	No	3	25	28			
	Quit	5	10	15			
Hypercholesterolemia	Yes	12	74	86	1.75	1	0.19
	No	3	7	10			
Diabetes mellitus	Yes	3	15	18	0.02	1	0.89
	No	12	66	78			
Obesity	Yes	2	11	13	0.15	1	0.70
	No	13	70	83			
Gender	Male	12	69	81	0.26	1	0.61
	Female	3	12	15			
Positive family history ^a	Yes	5	23	28	0.15	1	0.70
	No	10	58	68			

^a History of AMI, CAD or sudden cardiac death.

We further proceeded to investigate metabolic and individual lifestyle habits as confounding factors contributing to restenosis. However, we could not find any associations. In fact, 46 of the 53 smokers enrolled in our study did not develop restenosis. Smoking has not been associated with restenosis in some populations in Western Europe (38).

It seems that although men have a higher incidence of coronary heart disease, in our study, restenosis was observed in both genders at almost the same rate. In accordance with other studies (39), no association was observed between gender and restenosis, which could initially be attributed to the small number of women enrolled in the study. However, in contrast to the aforementioned study, hypertension was not related to restenosis – at least in Greeks with CAD – probably due to the lack of association of α_{2B} -AR polymorphism with hypertension (54). In addition, no significant differences were observed between diabetics and non-diabetics with regard to the rate of restenosis. Similarly to other studies (43), diabetes and hypercholesterolemia could not be used as indicators for restenosis. In contrast, low glucose and high serum high-density lipoprotein (HDL) levels in Norwegians and Irish patients were inversely related to the incidence of restenosis (39). On the other hand, poor glycemic control and vessel size have been shown to be independent predictors of restenosis in

patients with diabetes mellitus (44). In addition, advanced age, duration of diabetes mellitus and the number of diseased coronary vessels and segments may be other specific risk factors for restenosis (44). Some other studies have shown that infections such as *Chlamydia pneumoniae* could play a role in the mechanism of restenosis (45). Overall, major predictors for in-stent restenosis include final diameter of the stenosis, history of unstable angina rather than a more stable pattern of coronary heart disease, renal failure requiring hemodialysis, and vasospastic angina (39).

The lack of consistency in associating risk factors to restenosis in the literature probably reflects the complexity of the restenosis process itself, involving multi-locus influences and interactions. Individual genes may play a modest role in the pathogenesis of restenosis, and confounding factors, such as the individual, gender, ethnic origin, positive family history for AMI, CAD or sudden cardiac death, or smoking, may decrease the chance of identifying a causative relation between the genes responsible and the disease. Recently, seven single-nucleotide polymorphisms in genes involved in cardiovascular pathophysiology, inflammatory responses and cell-cycle control have been associated with post-PTCA restenosis (48) and point to the need to use multiple candidate gene approaches to enhance the prediction

Table 4 Assessment of genotype vs. possible confounding factors.

Variable		Genotype, n			Pearson's χ^2 values		
		I/I	I/D	D/D	χ^2	df	p
Hypertension	Yes	23	6	2	2.04	2	0.36
	No	47	17	1			
Hypercholesterolemia	Yes	63	20	3	0.53	2	0.77
	No	7	3	0			
Diabetes mellitus	Yes	15	3	0	1.51	2	0.47
	No	55	20	3			

value of genetic variation for the process of restenosis. At present, however, the need for identification of susceptibility-to-restenosis genes still poses a great challenge.

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