HOSPITAL CHRONICLES 2007, 2(4): 176–181

ORIGINAL ARTICLE

Genetic Polymorphisms: An Insertion/ Deletion Polymorphism of the α_{2B}-Adrenoceptor Gene as a Risk Factor for Acute Coronary Events

Mika Scheinin, MD, Paula Heinonen, MD, Amir Snapir, MD

Department of Pharmacology & Clinical Pharmacology, University of Turku and Turku University Hospital, FIN- 20520 Turku, Finland

KEY WORDS: *α_{2B}-adrenoreceptor polymorphism, coronary heart disease, acute myocardial infarction, sudden cardiac death, genotyping, genetic studies*

ABBREVIATIONS
AMI = acute myocardial infarction
AR = adrenoreceptor
CHD = coronary heart disease
SCD = sudden cardiac death

Correspondence to:
Mika Scheinin, MD
Department of Pharmacology and Clinical Pharmacology
University of Turku
Turku, Finland
E-mail: mika.scheinin@utu.fi

ABSTRACT

BACKGROUND: We have recently identified a variant form of the human α_{2B}-adrenoreceptor (AR) gene. Based on the coronary vasoconstrictive property of α_2-adrenoreceptors in humans, we hypothesized that the naturally occurring D (deletion) variant confers reduced receptor desensitization and therefore increased vasoconstriction in humans. This property could be associated with cardiovascular pathologies such as acute myocardial infarction (AMI) and sudden cardiac death (SCD). To test this hypothesis, we carried out two separate clinical genetic studies in middle-aged Finnish men, reported in two separate original publications.

METHODS: We examined a subset (912 men, aged 46 to 64 years) of the Kuopio Ischemic Heart Disease Risk Factor Study, an ongoing prospective population-based study investigating risk factors for cardiovascular diseases and related outcomes in men from eastern Finland (*Prospective Follow-up study*). Seven hundred out-of-hospital deaths of Finnish men in the Helsinki region were subjected to autopsy and formed the basis of the second, cross-sectional study investigating the associations between the α_{2B}-AR I/D polymorphism and coronary heart disease (*Autopsy study*). DNA for genotyping was extracted using standard methods. The α_{2B}-adrenoreceptor insertion/deletion (I/D) genotypes were determined using PCR-amplification and DNA electrophoresis.

RESULTS: Of the 912 subjects, 192 (21%) had the D/D genotype, 256 (28%) had the I/I genotype, and 464 (51%) were heterozygous. Thirty-seven acute coronary events occurred during the follow-up time: 15 in the D/D genotype group (7.8%), 12 in the I/D group (2.6%), and 10 in the I/I group (3.9%). Using a univariate analysis, the risk for an acute coronary event differed significantly between the three genotype groups (p=0.017). The D/D genotype group had a 3-fold increased risk for an acute coronary event in comparison with the I/D group (95% CI=1.4–6.5, p=0.004), and a non-significantly increased risk of 1.9 relative to the I/I genotype group (95% CI=0.8–4.1, p=0.128). In a Cox proportional hazards' model adjusting for several variables, the relative risk associated with the D/D genotype was 2.2 (95% CI=1.1–4.4, p=0.02). In the *autopsy cohort*, α_{2B}-AR I/D genotyping was accomplished for 683 men (98%). Of these, 152 (22%) had the D/D genotype, 186 (27%) had the I/I genotype, and 345 (51%) were heterozygous. The D/D genotype was associated with an increased risk for

Presented in part at the 4th International Cardiology Symposium "Cardiology Update" of Patras University, April 25-27, 2002

SCD and prehospital fatal AMI, with adjusted odds ratios (OR) of 1.8 - 2.4 compared to the other two genotypes ($p < 0.05$).

CONCLUSION: the D variant of the α_{2B}-AR gene is a causal risk factor for AMI and SCD in Finnish men. Further genetic epidemiological studies are still needed to confirm the causality and to test its generalization to other populations, and its physiological *in vivo* effects need to be identified.

INTRODUCTION

Three human α_2-adrenoceptor (α_2-AR) subtypes have been identified and named α_{2A}-AR, α_{2B}-AR, and α_{2C}-AR. Current drugs are not sufficiently α_2-AR subtype-selective, which complicates the investigation of the physiologic roles and therapeutic potential of the α_2-AR subtypes. New insight into the roles of the different α_2-AR subtypes in regulation of the cardiovascular system was recently achieved with strains of genetically engineered (knock-out, KO) mice deficient in either α_{2A}-AR or α_{2B}-AR or α_{2C}-AR.[1-3] The results of these studies suggest that the α_{2C}-AR subtype does not affect either blood pressure or vasoconstriction, that the α_{2A}-AR mediates the central hypotensive effects of clonidine-like α_2-AR agonists, and that the α_{2B}-AR mediates peripheral vasoconstriction.[4] The tissue distributions of the α_2-AR subtypes are not known in detail in humans, and the extent of α_{2B}-AR expression in vascular tissues is unknown. Some studies have failed to detect α_{2B}-AR expression in the aorta of experimental animals,[5,6] but others have reported that α_{2B}-ARs are expressed in rat vascular smooth muscle cells,[7] in human[8] and rat kidney,[9] and in central pathways of the autonomic nervous system.[10] A recent *in vivo* study in humans demonstrated that activation of α_2-ARs reduces coronary blood flow, in both normal and atherosclerotic arteries.[11]

We have recently identified a variant form of the human α_{2B}-AR gene.[12] The variant allele encodes a receptor protein with a deletion of 3 glutamate residues in an acidic stretch of 18 amino acids located in the third intracellular loop of the receptor. This acidic stretch is a unique feature in the primary structure of α_{2B}-AR in comparison to α_{2A}-AR and α_{2C}-AR. Sequence alignment of α_{2B}-AR polypeptides from different mammals reveals that the acidic stretch is conserved in mammalian α_{2B}-ARs, and that the acidic stretch is long in humans in comparison to other species. This suggests that the motif is important for the functionality of the receptor, and that the short form ("deletion", D) perhaps represents the ancestral form and the long form ("insertion", I) may represent a more recent allelic variant.

Negatively charged amino acids in the third intracellular loop of the α_{2A}-AR are important for receptor phosphorylation by β-adrenoceptor kinase,[13] and a similar role was considered possible for the negatively charged motif in α_{2B}-AR. Jewell-Motz and Liggett[14] used site-directed mutagenesis to delete and to substitute 16 amino acids of the stretch *in vitro*. The wild-type α_{2B}-AR underwent ~52% functional desensitization in transfected cells while agonist-promoted desensitization was ablated in both mutated receptors. The mutated receptors underwent agonist-promoted phosphorylation at levels of only 45-50% relative to the wild-type α_{2B}-AR. The same group also showed that the naturally occurring polymorphic D variant similarly impairs agonist-promoted receptor desensitization.[15] Thus, the natural polymorphism determines the susceptibility of the receptor to modulation by a key mechanism of dynamic regulation.

Based on the coronary vasoconstrictive property of α_2-ARs in humans, the peripheral vasoconstrictive property of the α_{2B}-AR subtype in mice, and the significance of this acidic region for the desensitization of the receptor, we hypothesized that the naturally occurring D variant confers reduced receptor desensitization and therefore increased vasoconstriction in humans. This property could be associated with cardiovascular pathologies such as myocardial infarction and sudden cardiac death. To test this hypothesis, we carried out two separate clinical genetic studies in middle-aged Finnish men, reported in two separate original publications[16] (Snapir et al, unpublished). Both studies had appropriate Ethics Committee approval.

MATERIALS AND METHODS

PROSPECTIVE FOLLOW-UP STUDY

We examined a subset of the Kuopio Ischemic Heart Disease Risk Factor Study, an ongoing prospective population-based study investigating risk factors for cardiovascular diseases and related outcomes in men from eastern Finland.[17] This region is known for its homogenous population[18] and high coronary morbidity and mortality rates.[19] Our study was based on a representative population sample of 912 men, aged 46 to 64 years at the start of follow-up. All subjects gave their written informed consent. The average time of follow-up was 4.5 years (range 1 to 7.6 years).

To assess the occurrence of acute coronary events, diagnostic information was collected from the Finnish hospital discharge registry, which covers all inpatient admissions into all hospitals and district health centers in Finland. These data were classified by an internist using WHO MONICA criteria. An acute coronary event was defined either as a definite acute myocardial infarction (AMI) or a possible AMI, based on

ECG, clinical, laboratory or autopsy findings, or as an episode of acute chest pain of >20 min without diagnostic ECG or enzyme findings, but requiring hospitalization (prolonged chest pain). Only first events were analyzed; a subject was excluded from further analysis when an acute coronary event had occurred.

AUTOPSY STUDY

Seven hundred out-of-hospital deaths of Finnish men in the Helsinki region were subjected to medico-legal autopsy and formed the basis of the second, cross-sectional study investigating the associations between the α_{2B}-AR I/D polymorphism and CHD. The material included all prehospital first-attack AMI deaths in the source population.[20] The methods used in diagnosis of AMI, measurement of coronary narrowing and coronary atherosclerosis, and assessment of risk factors for CHD and SCD have been described.[20]

DNA EXTRACTION AND ANALYSIS

DNA for genotyping was extracted using standard methods. The α_{2B}-AR insertion/deletion (I/D) genotypes were determined using PCR-amplification and DNA electrophoresis. Identification of the I and D alleles was based on their 9 bp size difference. A few samples of each genotype were sequenced to confirm the PCR results. The region of interest was amplified using a sense primer 5'-AGG-GTG-TTT-GTG-GGG-CAT-CT-3' and an anti-sense primer 5'-CAA-GCT-GAG-GCC-GGA-GAC-ACT-3', yielding products sized 112 bp (I) and 103 bp (D). To prevent observer bias, the investigators who collected the clinical data were blind to the subjects' genotypes and the investigator who performed the genotyping was blind to all subject information. The genotyping result of each subject was insertion/insertion (I/I, which is a homozygous form of the published α_{2B}-AR sequence,[21] deletion/deletion (D/D, which is a homozygous form of the recently discovered variant) or insertion/deletion (I/D).

STATISTICAL ANALYSIS

To assess the possible association of the α_2-AR I/D polymorphism with the risk for an acute coronary event in the follow-up study population, univariate Cox regression analysis was performed. Next, multivariate Cox regression analysis was performed to assess the impact of possible confounding and modifying factors on the observed association. Variables that were associated ($p < 0.10$ in the above analyses) with the α_{2B}-AR I/D genotype were entered into a multivariate Cox regression model. The same procedure was performed for all variables that were previously identified as risk factors for CHD in this study population. The variables identified in these two models were entered together with all established major risk factors for CHD[22] into a new multivariate Cox regression model. Logistic regression was used to analyze interactions between the α_{2B}-AR I/D variation and known risk factors for CHD. In the autopsy study, analyses of the effect of genotype on the odds of AMI with and without thrombosis, and comparisons between acute thrombosis cases and other SCD victims were based on logistic regression with and without the risk factor data. The interactions between genotype, age, and cause of death were also analyzed using logistic regression with Scheffe's post-hoc test.

RESULTS

PROSPECTIVE FOLLOW-UP STUDY

Of the 912 subjects, 192 (21%) had the D/D genotype, 256 (28%) had the I/I genotype, and 464 (51%) were heterozygous. Thirty-seven acute coronary events occurred during the follow-up time: 15 in the D/D genotype group (7.8%), 12 in the I/D group (2.6%), and 10 in the I/I group (3.9%). Of these 37 acute coronary events, 18 were classified as definite AMI, 12 as possible AMI and 7 as prolonged chest pain. Using a univariate Cox regression model, the risk for an acute coronary event differed significantly between the three genotype groups ($p=0.017$). The D/D genotype group had a 3.0 times increased risk for an acute coronary event in comparison with the I/D group (95% CI=1.4–6.5, $p=0.004$), and a non-significantly increased risk of 1.9 relative to the I/I genotype group (95% CI=0.8–4.1, $p=0.128$). The relative risk of the I/D genotype group was not significantly different from that of the I/I group (RR=0.6, 95% CI=0.3–1.4, $p=0.251$). We also applied the same analysis only to events classified as definite or possible AMI, excluding the prolonged chest pain category of the FINMONICA coronary event classification (30 events). In this analysis (overall $p=0.002$), the relative risk of the D/D group was significantly different from the I/D (RR=4.1, 95% CI=1.8–9.3, $p=0.0009$) and the I/I groups (RR=3.1, 95% CI=1.2–8.0, $p=0.02$). The risk ratio between the I/D and I/I groups was not significantly different from unity (RR=0.8, 95% CI=0.3–2.1, $p=0.6$). These results suggest that the D allele confers its effect on the risk for coronary events in a recessive mode of inheritance.

In a univariate Cox regression model, the D/D genotype was associated with an acute coronary event risk of 2.5 (95% CI=1.3–4.8, $p=0.006$) relative to the combined I/D + I/I group. A Kaplan-Meier survival function of event-free time (Fig. 1) illustrates the consistent difference in incidence of acute coronary events in the D/D group compared to the I/D + I/I group. A Cox regression analysis limited to the definite or possible AMI cases, excluding the prolonged chest pain category, revealed a relative risk of 3.7 (95% CI=1.8–7.5, $p=0.0006$) for the D/D vs. the I/D + I/I group.

Eighty-seven variables were tested for their association with the α_{2B}-AR I/D polymorphism to explore possible confounding factors. The genotype groups were not different in

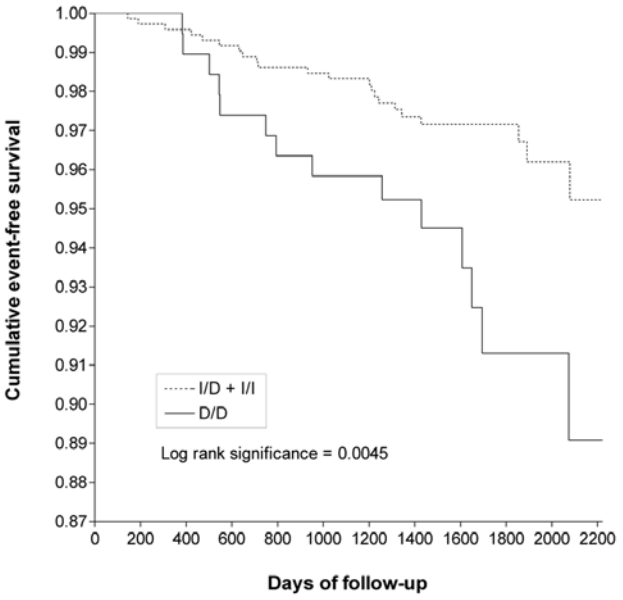

FIGURE 1.

terms of known major risk factors for CHD. [22] Only three variables were significantly different ($p < 0.05$) between the genotype groups. The D/D group had more acute coronary events. This group also had a slightly higher mean level of blood hemoglobin, and more common ischemic findings in exercise ECG and a lower mean four-day dietary cholesterol intake.[16] In addition, the α_{2B}-AR I/D polymorphism was non-significantly ($0.1 > p > 0.05$) associated with BMI (I/I> D/D >I/D). Thus, the blood hemoglobin concentration, ischemic findings in exercise ECG, the mean four-day dietary cholesterol intake, and BMI were introduced into a Cox regression model together with the variables that were previously described as AMI risk factors in this study population. Out of these variables, hypertension, LDL cholesterol level, and family history of CHD were identified by this model as independent AMI risk factors.

In a Cox proportional hazards' model adjusting for age, serum LDL and HDL cholesterol levels, smoking, hypertension, BMI, diabetes, and family history of CHD, the relative risk associated with the D/D genotype was 2.2 (95% CI=1.1–4.4, p=0.02). The relative risk was 3.2 (95% CI=1.5–6.7, p=0.002) when the analysis was performed using only the definite and the probable AMI events (n=30). When only men with no history of CHD were included in the analysis, the adjusted relative risk associated with the D/D genotype was 2.2 (95% CI=1.0–5.0, p=0.06)[16].

AUTOPSY STUDY

α_{2B}-AR I/D genotyping was accomplished for 683 men (98%). Of these, 152 (22%) had the D/D genotype, 186 (27%) had the I/I genotype, and 345 (51%) were heterozygous. No differences ($p > 0.1$) were found in major risk factors for CHD between the α_{2B}-AR I/D genotype groups. Multivariate analyses revealed that coronary narrowing percentages and areas of atherosclerotic lesions were similar in the D/D and in the I/I + I/D genotype groups suggesting that variation in this genetic locus was not involved in the development of coronary atherosclerosis. The D/D genotype was associated with an increased risk for SCD and prehospital fatal AMI, with adjusted odds ratios (OR) of 1.8 - 2.4 compared to the other two genotypes ($p < 0.05$). The I/D and the I/I genotypes were not different in respect to odds of SCD and AMI. When the data were analyzed assuming a recessive inheritance model, comparing the D/D genotype with the combined I/D + I/I genotypes, the D/D genotype again conferred an increased risk for SCD and AMI, especially for AMI without macroscopic thrombus (Table 1). The risks associated with the D/D genotype were considerably higher in a subpopulation of men who died suddenly before the age of 55.

DISCUSSION

Our prospective follow-up cohort study was the first to indicate that there is a strong statistical association between the D/D genotype of the α_{2B}-AR and an increased risk for acute coronary events. Men with the D/D genotype had 2.5 times the risk to experience an acute coronary event relative to men with the I/D and the I/I genotypes, independently of other major risk factors for CHD.

Our autopsy-based study confirmed that the D/D genotype of the α_{2B}-AR I/D variation is associated with an increased risk for AMI and SCD in Finnish men, especially in men who died before the age of 55. This association was largely due to the increased risk of the D/D genotype of non-thrombotic fatal prehospital AMI. Analysis of the coronary arteries revealed similar narrowing and other indices of atherosclerosis in the α_{2B}-AR I/D genotype groups. The study thus suggested that the α_{2B}-AR I/D polymorphism does not confer its increased risk for SCD and AMI through mechanisms directly related to progression of coronary atherosclerosis.

Our hypothesis that the D variant of the α_{2B}-AR gene confers increased vasoconstriction is based on two premises: the impaired desensitization associated with the D variant, and the role of the α_{2B}-AR subtype in vasoconstriction. The functional significance of this I/D polymorphism of the α_{2B}-AR gene was investigated in an *in vitro* cell culture model, and the results indicated that the D variant conferred impaired receptor desensitization under prolonged agonist activation.[15] The assumption that the α_{2B}-AR is critically involved in the regulation of vasoconstriction cannot be directly tested *in vivo* in humans, as subtype-selective α_{2B}-AR drugs are not (yet) available. Studies in KO mice have clearly documented a prominent role for the α_{2B}-AR in vasoconstriction. [1] Even

TABLE 1. α_{2B}-AR genotype frequencies in different causes of death and pathological findings, and the corresponding odds ratios. Data are presented for the entire study population and for a subpopulation of men younger than 55 years of age. Odds ratios were calculated in comparison with men dying of non-SCD and lacking evidence of old MI using multivariate analysis with genotype, age, BMI, smoking, alcohol consumption, diabetes, and hypertension as covariates

	N	DD	ID + II	OR (95% CI) DD vs. ID + II	P
All men					
Non-SCD	375*	21.3%	78.7%		
SCD	278	24.1%	75.9%	2.0 (1.2 to 3.5)	0.01
AMI	84	27.4%	72.6%	2.1 (1.1 to 4.1)	0.04
AMI with thrombus	39	20.5%	79.5%	1.5 (0.6 to 3.9)	0.2
AMI without thrombus	45	33.3%	66.7%	3.4 (1.4 to 8.1)	0.007
Men under 55					
Non-SCD	266	19.5%	80.5%		
SCD	107	26.2%	73.8%	4.5 (2.1 to 10)	<0.001
AMI	29	34.5%	65.5%	5.0 (1.8 to 14)	<0.001
AMI with thrombus	14	28.6%	71.4%	3.5 (0.8 to 14)	0.3
AMI without thrombus	15	40.0%	60.0%	14.9 (3.7 to 59)	<0.001

*SCD could not be confirmed or excluded in 30 men. AMI=acute myocardial infarction, BMI=body mass index, CI=confidence interval, DD=deletion/deletion, ID=insertion/deletion, II=insertion/insertion, OR=odds ratio, SCD=sudden cardiac death

if the perception of the α_{2B}-AR subtype as an important mediator of vasoconstriction is based on studies in KO mice, the similar blood pressure responses to α_2-AR agonists in humans and laboratory rodents[1] suggest that the α_{2B}-AR is a major mediator of vasoconstriction also in humans.

Augmentation in the constrictive properties of coronary arteries may be involved in the increased incidence of acute coronary events indirectly as the cause for intimal tearing leading to AMI or directly as in coronary artery spasm.[23,24] Increased vasoconstriction may lead to increased ischemic findings and coronary events in the D/D group by at least two additional mechanisms: 1. decreased coronary blood flow due to increased vasoconstriction of small coronary arteries and by 2. increased total peripheral resistance, causing increased cardiac work load and oxygen demand.

Our analysis did not disclose a mechanism by which the D/D genotype confers an increased risk for SCD and AMI. However, taking into account the role of α_{2B}-AR in vasoconstriction,[4] and that rupture of atherosclerotic lesions can induce rapid and marked increases in distal vascular resistance due to severe microvascular vasoconstriction,[25] our results may suggest that carriers of the D/D genotype are particularly prone to vasoconstriction in the vicinity of preexisting stenotic lesions in the epicardial coronary arteries or spasm of small-caliber coronary branches nourishing the subendocardium. These mechanisms may then contribute to the fatality of the ongoing coronary event.

CONCLUSIONS

The physiological functions of the α_{2B}-AR subtype in mice, the impaired desensitization properties of the human D variant *in vitro*, and our unbiased clinical genetic study designs and consistent results allow us to suggest that the D variant of the α_{2B}-AR gene is a causal risk factor for AMI and SCD in Finnish men. Further genetic epidemiological studies are still needed to confirm the causality and to test its generalization to other populations, and its physiological *in vivo* effects need to be identified. The lack of a proven *in vivo* mechanism weakens the strength of the associations and necessitates further studies on the physiological effects of this genetic variant on the human cardiovascular system.

REFERENCES

1. Link RE, Desai K, Hein L et al. Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science* 1996;273:803-805.
2. MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE. Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science* 1996;273:801-803.

3. Altman JD, Trendelenburg AU, MacMillan L et al. Abnormal regulation of the sympathetic nervous system in alpha2A- adrenergic receptor knockout mice. *Mol Pharmacol* 1999;56:154-161.
4. Kable JW, Murrin LC, Bylund DB. In vivo gene modification elucidates subtype-specific functions of alpha(2)-adrenergic receptors. *J Pharmacol Exp Ther* 2000;293:1-7.
5. Ping P, Faber JE. Characterization of alpha-adrenoceptor gene expression in arterial and venous smooth muscle. *Am J Physiol* 1993;265:H1501-H1509.
6. Handy DE, Johns C, Bresnahan MR, Tavares A, Bursztyn M, Gavras H. Expression of alpha2-adrenergic receptors in normal and atherosclerotic rabbit aorta. *Hypertension* 1998;32:311-317.
7. Richman JG, Regan JW. Alpha 2-adrenergic receptors increase cell migration and decrease F-actin labeling in rat aortic smooth muscle cells. *Am J Physiol* 1998;274:C654-C662.
8. Eason MG, Liggett SB. Human alpha 2-adrenergic receptor subtype distribution: widespread and subtype-selective expression of alpha 2C10, alpha 2C4, and alpha 2C2 mRNA in multiple tissues. *Mol Pharmacol* 1993;44:70-75.
9. Handy DE, Flordellis CS, Bogdanova NN, Bresnahan MR, Gavras H. Diverse tissue expression of rat alpha 2-adrenergic receptor genes. *Hypertension* 1993;21:861-865.
10. Tavares A, Handy DE, Bogdanova NN, Rosene DL, Gavras H. Localization of alpha 2A- and alpha 2B-adrenergic receptor subtypes in brain. *Hypertension* 1996;27:449-455.
11. Baumgart D, Haude M, Gorge G, et al. Augmented alpha-adrenergic constriction of atherosclerotic human coronary arteries. *Circulation* 1999;99:2090-2097.
12. Heinonen P, Koulu M, Pesonen U, et al. Identification of a three amino acid deletion in the alpha2B-adrenergic receptor that is associated with reduced basal metabolic rate in obese subjects. *J Clin Endocrinol Metab* 1999;84:2429-2433.
13. Onorato JJ, Palczewski K, Regan JW, Caron MG, Lefkowitz RJ, Benovic JL. Role of acidic amino acids in peptide substrates of the beta-adrenergic receptor kinase and rhodopsin kinase. *Biochemistry* 1991;30:5118-5125.
14. Jewell-Motz EA, Liggett SB. An acidic motif within the third intracellular loop of the alpha2C2 adrenergic receptor is required for agonist- promoted phosphorylation and desensitization. *Biochemistry* 1995;34:11946-11953.
15. Small KM, Brown KM, Forbes SL, Liggett SB. Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases G protein-coupled receptor kinase-mediated phosphorylation and desensitization. *J Biol Chem* 2001;276:4917-4922.
16. Snapir A, Heinonen P, Tuomainen TP, et al. An insertion/deletion polymorphism in the alpha2B-adrenergic receptor gene is a novel genetic risk factor for acute coronary events. *J Am Coll Cardiol* 2001;37:1516-1522.
17. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann Clin Res* 1988;20:46-50.
18. Sajantila A, Salem AH, Savolainen P, Bauer K, Gierig C, Paabo S. Paternal and maternal DNA lineages reveal a bottleneck in the founding of the Finnish population. *Proc Natl Acad Sci USA* 1996;93:12035-12039.
19. Keys A. Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease. Cambridge, Mass: Harvard University Press, 1980.
20. Mikkelsson J, Perola M, Penttilä A, Karhunen PJ. Platelet glycoprotein Ib? HPA-2 Met/VNTR B haplotype as a genetic predictor of myocardial infarction and sudden cardiac death. *Circulation* 2001;104:876-880.
21. Lomasney JW, Lorenz W, Allen LF et al. Expansion of the alpha 2-adrenergic receptor family: cloning and characterization of a human alpha 2-adrenergic receptor subtype, the gene for which is located on chromosome 2. *Proc Natl Acad Sci USA* 1990;87:5094-5098.
22. Grundy SM, Pasternak R, Greenland P, Smith SJ, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *Circulation* 1999;100:1481-1492.
23. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995;92:657-671.
24. Kaski JC, Tousoulis D, McFadden E, Crea F, Pereira WI, Maseri A. Variant angina pectoris. Role of coronary spasm in the development of fixed coronary obstructions. *Circulation* 1992;85:619-626.
25. Taylor AJ, Bobik A, Berndt MC, Ramsay D, Jennings G. Experimental rupture of atherosclerotic lesions increases distal vascular resistance: a limiting factor to the success of infarct angioplasty. *Arterioscler Thromb Vasc Biol* 2002;22:153-160.