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Original investigation

The insertion/deletion variation in the α_{2B}-adrenoceptor does not seem to modify the risk for acute myocardial infarction, but may modify the risk for hypertension in sib-pairs from families with type 2 diabetes

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Abstract

Background: An insertion/deletion polymorphism in the α_{2B}-adrenoceptor (AR) has been associated with the risk for acute myocardial infarction (AMI) and sudden cardiac death. In this study we tested whether this polymorphism is associated with the risk for AMI among members of families with type 2 diabetes.

Methods: 154 subjects with a history of AMI were matched for age and sex with one of their siblings who did not have a history of AMI. The prevalence of the genotypes of the α_{2B}-AR insertion/deletion polymorphism was compared between the siblings using McNemar's test. We also explored the data to see whether this genetic variation affects the risk for hypertension by using logistic regression models in the two subpopulations of subjects, with and without a history of AMI.

Results: Among all study subjects, 73 (24%) carried the α_{2B}-AR deletion/deletion genotype, 103 (33%) carried the insertion/insertion genotype, and 132 (43%) were heterozygous. The distribution of genotypes of the α_{2B}-AR insertion/deletion variation in the group of subjects with a history of AMI and their phenotype-discordant siblings did not statistically significantly differ from that expected by random distribution (p = 0.52): the deletion/deletion genotype was carried by 34 subjects with AMI (22%), and by 39 subjects without AMI (25%). Neither did we observe any significant difference in deletion allele frequencies of the α_{2B}-AR insertion/deletion polymorphism between patients with a history of AMI (0.44) and their sib-pair controls (0.46, p = 0.65). In an exploratory analysis, the α_{2B}-AR deletion/deletion genotype was associated with increased odds for hypertension compared with subjects carrying any of the other genotypes.

Conclusions: The deletion/deletion genotype of the α_{2B}-AR does not emerge in this study as a risk factor for AMI among members of families with type 2 diabetes; however, it might be involved in the development of hypertension.

Background

Vascular disease is the major cause of morbidity and mortality in patients with diabetes mellitus [1]. *In vivo* studies in humans have shown that α_2-adrenoceptors (ARs) mediate constriction of large and small coronary arteries [2], an effect augmented by atherosclerosis [3], and mediate peripheral vasoconstriction [4]. However, lack of α_2-AR subtype-selective drugs has precluded the clarification of the precise roles of each subtype in these responses to α_2-AR activation. Based on studies on genetically engineered mice lacking the α_2-AR A or B or C subtype, it has been demonstrated that the α_{2B}-AR subtype mediates peripheral vasoconstriction [5].

A variant form of the human α_{2B}-AR gene encodes a receptor protein with deletion of three glutamate residues [6]. Studies on transfected cells have revealed that this deletion variant manifests significantly impaired agonist-promoted receptor desensitization [7]. *In vivo* studies in humans have associated the deletion/deletion (DD) genotype with reduced flow-mediated dilatation of the brachial artery [8], and reduced coronary blood flow and increased peripheral resistance upon adrenaline infusion [9]. In a population-based, prospective study on 912 middle-aged men, the DD genotype was associated with an increased incidence of acute myocardial infarction (AMI) in comparison to the other two genotypes [10]. In another population of men who died suddenly outside of a hospital, the DD genotype was associated with increased relative risks for AMI and sudden cardiac death [11]. The relative risks for AMI and sudden cardiac death were especially high in men who died before the age of 55 [11].

To explore the possible effect of the insertion/deletion variation in the α_{2B}-AR gene on the risk for AMI in a population at high risk for type 2 diabetes and cardiovascular disease, we conducted a study on 154 sibling-pairs from Finland, discordant for AMI. We hypothesized that the DD genotype will be more prevalent among patients with a history of AMI than among their phenotype-discordant sib-pairs. We also explored the possibility that the DD genotype is associated with hypertension in this study population.

Methods

Study population

This study had a phenotype-discordant sib-pair design with AMI as the selection phenotype. 154 subjects (88 men, 66 women) from eastern [12] and western [13] Finland who reported having had an AMI were selected from families with type 2 diabetes participating in the Botnia study [13]. Each case-subject was matched for sex and age with one of his or her siblings who had not had an AMI. Characteristics of the study population are presented in Table 1. Before participating in the study, voluntary informed written consent was obtained from each subject. The study protocol was approved by the ethics committee of Lund University.

Using a multiple-risk-factor assessment equation [14], with complete data available for 79% of the study population, global risk-assessment scoring was calculated, and the age-stratified relative risk for coronary heart disease was estimated. The majority of the subjects (60%) had above average relative risk for coronary heart disease (26% moderately above average relative risk, 34% high relative risk), 27% had below average relative risk, and 13% had an average relative risk. Taking into account the relatively old age of the subjects in this population (44% over 70 years, 63% over 65 years), the absolute risk for coronary heart disease in this population may thus be considered high.

Phenotypic characterization of the study subjects

A standardized health questionnaire was filled by a trained nurse together with the subject, covering medical history, including current medication (use of any drug prescribed by a physician at the time of examination), and smoking and alcohol consumption habits. A subject was defined as a smoker if he or she had smoked for a period of at least one year.

Myocardial infarction was defined as an acute coronary event requiring hospitalization; the information was verified against the subject's hospital records.

A subject was classified as having hypertension if he or she had systolic blood pressure (BP) ≥ 160 mmHg, or diastolic BP ≥ 90 mmHg, or was treated with antihypertensive medication [15]. Diagnosis of type 2 diabetes mellitus was based upon the WHO criteria of 1998 [15].

Measurements and assays

BP was measured three times from the right arm at 5 min intervals with the subject in seated position after a 30 min rest, and the mean of the three values was used. This procedure followed well established routines of how to measure BP [16].

Table 1: Characterization of the study population by AMI phenotype and the α_{2B}-adrenoceptor insertion/deletion genotype.

	Phenotype			Genotype					
	AMI (n = 154)	**No AMI (n = 154)**	**P**	**DD (n = 73)**	**ID (n = 132)**	**II (n = 103)**	**P**	**ID + II (n = 235)**	**P (vs. DD)**
Age (years)	67.1 ± 0.8	66.9 ± 0.8	0.59	66.7 ± 1.0	67.0 ± 0.8	67.2 ± 1.0	0.35	67.1 ± 0.6	0.45
BMI (kg/m^2)	28.0 ± 0.3	27.2 ± 0.3	0.038	28.1 ± 0.5	27.2 ± 0.4	27.9 ± 0.5	0.62	27.5 ± 0.3	0.43
Systolic BP (mmHg)	143 ± 1	145 ± 2	0.38	145 ± 2	144 ± 2	143 ± 2	0.81	144 ± 1	0.56
Diastolic BP (mmHg)	80.0 ± 0.9	82.1 ± 1.0	0.063	80.8 ± 1.1	81.7 ± 1.0	80.2 ± 1.2	0.62	81.1 ± 0.8	0.94
LDL cholesterol (mmol/l)	3.71 ± 0.09	3.76 ± 0.08	0.44	3.82 ± 0.11	3.70 ± 0.10	3.71 ± 0.10	0.84	3.71 ± 0.07	0.55
HDL cholesterol (mmol/l)	1.13 ± 0.02	1.27 ± 0.03	<0.001	1.19 ± 0.02	1.18 ± 0.03	1.19 ± 0.04	0.57	1.20 ± 0.03	0.32
Hypertension	127 (89%)	101 (70%)	<0.001	60 (53)	96 (97)	72 (78)	0.049	168 (175)	0.023
Smoking	76 (56%)	50 (38%)	<0.001	25 (29)	57 (55)	44 (42)	0.75	101 (97)	0.47
Diabetes	115 (75%)	90 (58%)	0.002	47 (49)	87 (88)	71 (69)	0.80	158 (156)	0.53

Data for continuous variables are presented as mean ± SEM. Discrete variables are presented as number of subjects, and in parenthesis, either percentage of the group for which data are available (AMI phenotype) or the expected number by random distribution (α_{2B}-adrenoceptor insertion/deletion genotype). Paired *t* test and McNemar's test were used to calculate the p value comparing the siblings discordant for history of AMI. Linear mixed models were used to calculate the p values in the comparison of the genotype groups.

Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured on a Cobas Mira analyzer (Hoffman LaRoche, Basel, Switzerland). The LDL cholesterol concentrations were calculated using the Friedewald formula.

Genotyping

Genomic DNA was extracted from whole blood using standard methods. The method used to genotype the α_{2B}-AR insertion/deletion polymorphism was based on PCR amplification and DNA electrophoresis, and has been described elsewhere [10].

Statistical analyses

Paired *t* tests (for continuous variables) and McNemar's test (for discrete variables) were used in the characterization of the population. Since the study population was sampled as sibling pairs, analysis of the entire population according to the three genotypes must take into account the possibility that error terms are not independent but are correlated between siblings. We therefore used linear mixed models with the genotype group information as a fixed factor, and the sib-pair information as a random factor to characterize the genotype groups. The effect of the α_{2B}-AR insertion/deletion polymorphism on the risk for AMI was estimated by the difference in the frequency of the DD genotype between the phenotype-discordant sibling pairs, and was tested using McNemar's test. Odds ratios for hypertension and their 95% confidence intervals (95% CI) were calculated using logistic regression models. Using a univariate logistic regression model, the effect of the different genotypes of the α_{2B}-AR insertion/deletion variation on the odds for hypertension was explored. In multivariate logistic regression models, age, sex, smoking, diabetes, body mass index, serum LDL cholesterol, and the α_{2B}-AR genotype were added as covariates in a conditional stepwise fashion with p = 0.05 for entry and 0.1 for removal. Statistical computations were performed with SPSS/Win version 11.0.1 software (SPSS Inc., Chicago, IL, USA).

Results

Genotype information was obtained for all 308 subjects. Of these, 73 (24%) carried the DD genotype, 103 (33%) the insertion/insertion (II) genotype, and 132 (43%) the insertion/deletion (ID) genotype. Except for hypertension, no differences (p > 0.1) in major risk factors for coronary heart disease were found between the α_{2B}-AR insertion/deletion genotype groups (Table 1).

The prevalence of the DD genotype did not statistically significantly differ between subjects with a history of AMI and their sib-pair controls (p = 0.5): the DD genotype was carried by 34 subjects with AMI (22%), and by 39 subjects without AMI (25%). Neither did we observe any significant difference in D allele frequencies of the α_{2B}-AR insertion/deletion polymorphism between patients with a history of AMI (0.44) and their sib-pair controls (0.46, p = 0.65).

To explore the possible association of the α_{2B}-AR insertion/deletion variation and hypertension, we compared the prevalence of hypertension in the genotype groups. In subjects with no history of AMI, the DD genotype was associated with increased odds for hypertension when compared with the II genotype group (odds ratio (OR) 3.7, 95% CI 1.2–11.1, p = 0.021), or when compared with the II + ID genotype groups combined (OR 3.2, 95% CI 1.2–8.9, p = 0.026) (Table 2). Among subjects with AMI, there were only 16 individuals who were not classified as hypertensive according to the employed criteria, and no

Table 2: Odds ratios for hypertension in relation to the α_{2B}-adrenoceptor genotype and other cardiovascular risk factors.

N (hypertensives)	AMI 143 (127)		No AMI 144 (101)	
	OR (95% CI)	P	OR (95% CI)	P
Univariate models				
α_{2B}-AR D vs. I	1.6 (0.6–4.7)	0.36	1.8 (0.9–3.7)	0.12
α_{2B}-AR ID vs. II	1.4 (0.4–4.1)	0.59	1.3 (0.6–2.8)	0.53
α_{2B}-AR DD vs. II	2.6 (0.5–13.2)	0.26	3.7 (1.2–11.1)	0.021
α_{2B}-AR DD vs. II + ID	2.2 (0.5–10)	0.36	3.2 (1.2–8.9)	0.026
Age	1.0 (0.9–1.0)	0.79	1.1 (1.0–1.1)	0.008
BMI	1.1 (1.0–1.4)	0.037	1.1 (1.0–1.2)	0.038
LDL cholesterol	1.3 (1.0–1.8)	0.059	1.5 (1.0–2.2)	0.032
Smoking	0.6 (0.2–2.0)	0.43	0.5 (0.2–1.2)	0.11
Diabetes	1.9 (0.6–5.8)	0.29	1.3 (0.6–2.7)	0.48
Multivariate model*				
α_{2B}-AR DD vs. II + ID	N/A		4.0 (1.2–13.3)	0.024
Age	N/A		1.1 (1.0–1.1)	0.020
BMI	N/A		1.2 (1.0–1.4)	0.006
LDL cholesterol	N/A		1.8 (1.1–3.0)	0.032

Results are from univariate and conditional multivariate logistic regression models. *Data are presented for the last step of the stepwise conditional insertion of variables into the models. Adjustment was done for age, sex, smoking, diabetes, BMI, and LDL cholesterol, which were inserted into a multivariate logistic regression model in a stepwise manner (p = 0.05 for entry and 0.1 for removal). N/A denotes not applicable (all the variables were rejected by the models). I, insertion; D, deletion; BMI, body mass index

Table 3: Blood pressure (mmHg) of subjects not treated with an antihypertensive drug, according to α_{2B}-AR I/D genotypes

	AMI						No AMI					
	DD (n = 3)	ID (n = 12)	II (n = 8)	P*	ID + II (n = 20)	P*	DD (n = 14)	ID (n = 26)	II (n = 32)	P*	ID + II (n = 58)	P*
Systolic BP	144 ± 9	144 ± 3	130 ± 6	0.090	139 ± 3	0.54	154 ± 8	136 ± 4	146 ± 4	0.043	141 ± 3	0.055
Diastolic BP	86 ± 5	83 ± 3	77 ± 3	0.28	81 ± 2	0.43	86 ± 4	77 ± 2	82 ± 2	0.067	80 ± 1	0.065

*One-way ANOVA was used in the comparison of BP between the three genotype groups; *t* test was used in the comparison of BP of carriers of the DD genotype with carriers of the other genotypes combined. I, insertion; D, deletion

statistically significant associations were found between the α_{2B}-AR genotypes and hypertension.

In subjects that were not treated with an antihypertensive drug, a trend for increased systolic and diastolic BP was observed among those carriers of the α_{2B}-AR DD genotype who had not had AMI (Table 3).

Discussion

The main finding of this study is a lack of association between the α_{2B}-AR DD genotype and AMI among members of families with type 2 diabetes. Another finding of this study is a possible association between this genotype and hypertension.

Association with AMI

Earlier, association of the α_{2B}-AR DD genotype with AMI was reported in a population-based prospective study on 912 middle-aged men from eastern Finland [10], and in a series of 700 unselected sudden out-of-hospital deaths of Finnish men subjected to medico-legal autopsy [11]. The populations in these studies were younger than the population of the current study, and their relative risk for coronary heart disease was expected to be similar to the general population. The lack of association between the α_{2B}-AR DD genotype and AMI in high-risk subjects observed in the present study may suggest that the mechanism by which the α_{2B}-AR deletion variant confers its observed increased risk for AMI is not directly dependent on established coronary heart disease risk factors that lead to atherosclerosis, and that in subjects at high risk for coronary heart disease the effect of this genetic variation is

diluted. This interpretation was supported by the morphometric autopsy findings of the sudden death study [11]. In that study [11], the DD genotype was associated with increased odds for AMI and sudden cardiac death, but not with the severity of coronary atherosclerosis.

Association with hypertension and BP

In subjects that had not had an AMI, the DD genotype was associated in univariate logistic regression analysis with increased odds for hypertension. However, since this analysis was conducted as further exploration of the data rather than based on an *a priori* hypothesis, adjustment for multiple testing should be employed. A corrected p value corresponding to 0.05 when testing 2 independent hypotheses would be 0.0253 (based on the equation $\alpha = 1 - 0.95^{1/N}$ where N is the number of hypotheses tested). The observed p value for the association between the DD genotype and hypertension (about 0.02) has therefore only borderline statistical significance.

Two previous studies explored the possible involvement of the α_{2B}-AR insertion/deletion variation in the development of hypertension, and reported lack of association [10,18]. However, in the study on 912 middle-aged men [10], the prevalence of cardiovascular risk factors was different from that of the current study, making it difficult to compare the results of the studies. Because of the very low frequency of the DD genotype (n = 3) among the subjects in the study on 155 sib-pairs concordant for hypertension [18], that study can be considered to have insufficient power to detect linkage between the α_{2B}-AR DD genotype and the studied phenotype.

Several earlier studies have suggested a role for the α_{2B}-AR in the development of hypertension. α_2-adrenergic vasoconstriction in humans [4] is probably mainly mediated by the α_{2B}-AR subtype [5], and the observed decreased agonist-promoted desensitization property of the deletion variant [7] may suggest that the DD genotype confers increased vasoconstriction that leads to increased peripheral resistance – a common finding in hypertension [19]. Furthermore, rodent studies suggest a significant role for the α_{2B}-AR in acquired and hereditary hypertension [20-22]. Additionally, loci on human chromosome 2 have been linked with increased BP and hypertension in several recent genome-wide studies [23,24]. So far, the location of the human α_{2B}-AR gene (ADRA2B, GeneBank accession number M34041) on chromosome 2 has been tentatively placed at 2p13-q13, but more precise mapping will be needed to confirm or exclude the α_{2B}-AR gene as the hypertension risk gene involved in these genome-wide linkage study results.

Methodological considerations

Using a health questionnaire, a subject was categorized as a smoker if he or she had smoked for a period of at least one year. This information may be considered insufficient to provide a complete estimation of the impact of this risk factor on the total risk for AMI.

Interpretation of results from studies with a case-control design, where the studied risk factor may also affect survival, such as in the present study, is not straightforward. Inherently by the study design, only survivors of an acute coronary event were included as index cases in the present study – possibly creating selection bias. Such an effect was demonstrated by Hamajima *et al.* [17], who showed that the effects of genotypes increasing disease risk and fatality rate are underestimated, while those increasing the risk and improving prognosis are overestimated.

It should also be acknowledged that the apparently unaffected sibs were themselves at higher risk for AMI on the basis of family history, both by belonging to high-risk type 2 diabetes families and by having a sib with AMI. Thus, the unaffected sib may also become affected in a short time.

Conclusions

To conclude, the results of this study suggest that the α_{2B}-AR insertion/deletion variation either does not play a significant role in AMI morbidity in members of families with type 2 diabetes that are at high risk for cardiovascular diseases, or that by affecting mortality, the DD genotype could be underrepresented among the surviving AMI cases. We also propose a role for this genetic variation in the development of hypertension; however, further studies are required to replicate this finding.

Abbreviations

α_2-AR, α_2-adrenoceptor; AMI, acute myocardial infarction; BMI, body mass index; BP, blood pressure; CI, confidence interval; DD, deletion/deletion; HDL, high density lipoprotein; ID, insertion/deletion; II, insertion/insertion; LDL, low density lipoprotein; OR, odds ratio; PCR, polymerase chain reaction

Competing interests

We declare that no one of us has competing interests in connection with this paper.

Authors' contributions

AS conceived the study, participated in the design of the study, performed the statistical analysis, and drafted the manuscript. MOM participated in the design of the study and carried out the molecular genetic studies. MS and LCG participated in the design of the study and its coordi-

nation. All authors read and approved the final manuscript.

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