The β_3-Adrenergic Receptor Trp64Arg Mutation Is Not Associated With Coronary Artery Disease

Karl Stangl, Ingolf Cascorbi, Michael Laule, Verena Stangl, Christian Meisel, Klaus-Dieter Wernecke, Sabine Ziemer, Gert Baumann, Ivar Roots, and Hans Hauner

There is some evidence that the Trp64Arg polymorphism of the β_3-adrenergic receptor (β_3-AR) is associated with atherogenic risk factors that include weight gain, insulin resistance, and diabetes. The objective of this cross-sectional study was to investigate the relationship between the Trp64Arg polymorphism and coronary artery disease (CAD). A total of 1,000 consecutive patients with angiographically confirmed CAD and 1,000 controls, carefully matched for age and sex, were genotyped for the Trp64Arg polymorphism by polymerase chain restriction and subsequent restriction fragment length polymorphism analysis. Among cases with CAD, 83.3% were wild-type *Trp/Trp*, 15.8% were heterozygotes, and 0.9% were homozygous *Arg/Arg* compared with 82.3%, 17.3%, and 0.4%, respectively, among controls ($P = .27$). The odds ratios for the presence of *Trp/Arg* and *Arg/Arg* in cases and controls were 0.90 (95% confidence interval [CI] 0.7 to 1.2; $P = .40$) and 2.2 (95% CI 0.7 to 7.2; $P = .17$), respectively. There was no effect modification by gender and atherogenic risk factors, including diabetes, hypercholesterolemia, hypertension, and smoking. Furthermore, there was no evidence of an association with premature disease onset (<40 years) or extent of disease. In conclusion, the results of this study in a large sample of clinically well-characterized patients indicate that neither the *Trp/Arg* nor the *Arg/Arg* genotype represents a major risk factor for angiographically confirmed coronary artery disease.
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THERE IS SOME evidence that a mutation of the human β_3-adrenergic receptor (β_3-AR) gene, characterized by substitution of arginine for tryptophan at position 64 (Trp64Arg), is associated with an increased capacity to gain body weight, features of insulin resistance, and early onset of type 2 diabetes mellitus,[1-3] all considered to be cardiovascular risk factors.[4] Although most studies suggest that *Trp/Arg* has no major impact on atherogenic risk factors,[5-20] there are some reports that the *Arg/Arg* genotype is associated with more distinct metabolic disturbances.[1,7,15,19] However, linkage of *Arg/Arg* to features of the insulin resistance syndrome is not undisputed.[21] Because the β_3-AR plays an important role in catecholamine-induced lipolysis,[22] and some studies report that its mutation at position 64 is linked to atherogenic risk factors,[2,3,5-7,15] the question arises whether the Trp64Arg polymorphism has an impact on the development of coronary artery disease (CAD).

Many studies of the β_3-AR gene polymorphism have been performed with regard to obesity and diabetes (for review see Allison et al[13] and Fujisawa et al[14]). In contrast, little is known about the possible role played by this polymorphism in other diseases, especially those that are consequences or complications of metabolic disturbances such as atherosclerosis and, in particular, CAD. Until now only three studies (with relatively small numbers of CAD patients enrolled) have investigated the potential role of the Trp64Arg mutation as a putative risk factor. In a cohort of 271 middle-aged white Americans with incident CAD,[23] as well as in 185 nondiabetic Finnish subject,[24] the Trp64Arg polymorphism failed to predict CAD. Similarly, the Trp64Arg polymorphism was not associated with CAD in 428 Japanese subjects.[25] With regard to the reported frequencies of 10.8% for *Trp/Arg* and 0.8% for *Arg/Arg* in Central European whites,[21] the comparatively small sample sizes of these studies do not allow definite assessment of the functional importance of both *Trp/Arg* and *Arg/Arg* genotypes. In general, association studies are exposed to the risk of bias, and large samples, in addition to adequate clinical characterization of the subjects, are accordingly necessary for such investigations. Consequently, several hundred or more participants are required to obtain an adequately narrow confidence interval for the odds ratio.[26] To satisfy these essential requirements, we designed a cross-sectional case-control study with large sample size (2,000 subjects), in which we investigated the importance of the Trp64Arg polymorphism of the β_3-AR gene as a putative atherogenic risk factor.

SUBJECTS AND METHODS

The design of the study has been described in detail elsewhere.[27] Between October 1995 and January 1997, we recruited 1,000 consecutive white patients from the Berlin area who had been admitted for angiography for suspected CAD and/or for elective or emergency interventions at the Charité University Medical Center in Berlin. An additional 1,000 patients also admitted to this hospital served as controls. They were matched by age ($\pm$3 years), sex, and time of admittance (within 2 weeks). Exclusion criteria included clinical evidence of coronary or peripheral artery disease or of any form of vasculitis. We further excluded all severe disease states potentially affecting the atherogenic risk factor profile and/or coagulation status. For the purpose of this study, all controls were screened on the basis of patient history, physical examination, electrocardiogram, and echocardiogram. All participants had given their written consent according to the study protocol approved by the ethical committee of the Charité Hospital in 1994.

From the Medizinische Klinik und Poliklinik, Institut für Klinische Pharmakologie, Institut für Medizinische Biometrie, Institut für Pathologische und Klinische Biochemie, and Deutsches Diabetes Forschungsinstitut an der Heinrich Heine, Universität Düsseldorf, Düsseldorf, Germany.

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Address reprint requests to Karl Stangl, MD, Medizinische Klinik und Poliklinik, Kardiologie, Charite', Campus Mitte, Schumannstr 20/21, 10117 Berlin, Germany.

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Operational Definitions

CAD was defined as stenosis ≥ 50% in a major coronary artery or a major branch. The severity of CAD was classified according to the number of affected arteries: ie, as 1-, 2-, or 3-vessel disease. Diagnosis of myocardial infarction was established both by reference of patient case notes and by angiographic findings. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (kg/m^2). Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference (cm/cm). Subjects were defined as smokers if they currently smoked or had smoked within the previous 10 years. Hypertension was defined as blood pressure ≥ 140/90 mm Hg and/or history of hypertension and/or use of antihypertensive drugs. Hypercholesterolemia was defined as a total cholesterol level ≥ 6.46 mmol/L. Diabetes was defined as fasting plasma glucose ≥ 140 mg/dL and/or a history of diabetes and/or use of antidiabetic drugs.

Analytical Methods

Blood was collected in the morning after an overnight fast of at least 10 hours. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany). LDL cholesterol, apolipoprotein (apo) A1, and apo-B were determined by commercially available immunoturbidimetric assays (Tina-quant; Boehringer Mannheim). Fasting plasma glucose was established enzymatically using the hexokinase method.

Trp64Arg Genotyping

DNA was extracted from peripheral blood by standard methods.[28] The T/C point mutation leading to a *Trp/Arg* exchange in codon 64 of the β_3-AR gene was evaluated by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). Primers were designed according to the sequence of van Spronsen et al.[29] A 192-bp fragment taken from exon 1 of the β_3-AR gene was amplified using 0.5 units of AmpliTaq Gold (Perkin Elmer, Weiterstadt, Germany), 0.2 mmol/L deoxynucleotides, 0.2 μmol/L downstream-primer β_3-1 5′-GGC AAC CTG CTG GTC ATC, 0.2 μmol/L upstream-primer β_3-2 5′-GTC CAC CGA GGT CCA CAG, and 1.0 mmol/L $MgCl_2$ in a final volume of 25 μL. PCR conditions were 45 cycles for 30 seconds at 94°C, 30 seconds at 57°C, and 30 seconds at 72°C using a Perkin-Elmer GeneAmp 9600 or 9700 thermocycler. PCR efficiency was checked on a 2% agarose gel for 20 minutes at 120 V. Fifteen microliters of the amplified product was incubated with 90 units of *Msp*I (New England Biolabs, Schwalbach, Germany), in a final volume of 25 μL, at 37°C overnight. Fragments were separated on 3.5% NuSieve 3:1 gel (Biozym, Hessisch Oldendorf, Germany) for 75 minutes at 100 V. The wild type presented a constitutive restriction site, resulting in a 118- and a 74-bp fragment, whereas mutated alleles presented 3 fragments of 88, 74, and 30 bp. A genotype was not available in 8 cases or 12 controls for technical reasons.

Statistical Analysis

The design of the study was matched case-control, with 1,000 subjects in each group, as described in detail elsewhere.[27] Values are presented as medians and as 25th and 75th percentiles. We applied the Mann-Whitney *U* test and adjusted the results with the help of linear multiple regression analysis. Allelic and genotype frequencies were determined from observed genotype counts. The Hardy-Weinberg equilibrium was assessed by χ^2 test. We performed logistic regression analysis, adjusted for age, sex, BMI, diabetes, hypertension, hypercholesterolemia, and smoking, to evaluate the influence of the β_3-AR genotypes on the following as dependent variables: early disease manifestation (group comparisons: age of manifestation <40 years *v* ≥40 years); acute myocardial infarction (group comparison: yes *v* no), or previous myocardial infarction (group comparison: history yes *v* history no); and disease severity (1-vessel *v* 3-vessel disease). Finally, we tested the importance of established cardiovascular risk factors and β_3-AR genotypes as independent risk factors for CAD by using logistic regression analysis adjusted for age and sex.

RESULTS

Table 1 summarizes the clinical characteristics of cases and controls, including risk factors for CAD. Both groups differed significantly with respect to the prevalence of diabetes, hypercholesterolemia, hypertension, and smoking, as well as levels of triglycerides, total cholesterol, LDL cholesterol, apo-A1, and apo-B.

Among cases, 826 (83.3%) were wild type (*Trp/Trp*), 157 (15.8%) were heterozygotes, and 9 (0.9%) were homozygous *Arg*. The corresponding values among controls were 813 (82.3%), 171 (17.3%), and 4 (0.4%), respectively, and did not differ significantly.

Genotype frequencies were consistent with the Hardy-Weinberg equilibrium. The observed (expected) frequencies were *Trp/Trp*, 83.3% (83.1%); *Trp/Arg*, 15.8% (16.1%); and *Arg/Arg*, 0.9% (0.8%) for the CAD group ($P = .97$) and *Trp/Trp*, 82.3% (82.7%); *Trp/Arg*, 17.3% (16.5%); and *Arg/Arg*, 0.4% (0.8%) for the control group ($P = .46$).

Neither the *Trp/Arg* (odds ratio [OR] 0.90, 95% confidence interval [CI] 0.7 to 1.2; $P = .40$) nor the *Arg/Arg* genotype (OR 2.2; 95% CI 0.7 to 7.2; $P = .17$) were significantly more frequent among cases than in controls; the OR of *Arg* allele carriers (*Trp/Arg* + *Arg/Arg*) was 0.93 (95% CI 0.7 to 1.2; $P = .56$). Stratification with respect to gender, hypertension, and diabetes showed a trend to higher frequencies of *Arg/Arg* in these subgroups without achieving statistical significance.

In a further step, we performed logistic regression to evaluate the importance of the mutation for subgroups of cases at higher risk, including those with early manifestation of the disease (<40 years), history of acute or chronic myocardial infarction, and 3-vessel disease. The results disclosed no significant influence of either *Trp/Arg* or *Arg/Arg* genotype on these characteristics (data not shown).

After adjusting for sex and age, we compared the three genotypes, as well as carriers and noncarriers of the *Arg* allele (*Trp/Trp v Trp/Arg* + *Arg/Arg*), with regard to a number of clinical variables, including the following: BMI; WHR; systolic and diastolic blood pressure; triglyceride level; total, HDL, and LDL cholesterol levels; apo-A1 and –B levels; and age of disease manifestation. The only significant difference was observed in controls: the four *Arg/Arg* subjects had lower total cholesterol levels than *Trp/Trp* subjects (4.1, 95% CI 3.8 to 5.2 mmol/L, *v* 5.5, 95% CI 5.1 to 6.6 mmol/L; $P = .036$). However, comparison of carriers and noncarriers of the *Arg* allele resulted in diminution of the differences (5.56, 95% CI 4.7 to 6.4, *v* 5.5, 95% CI 5.1 to 6.6 mmol/L; $P = .40$). Although these differences were not statistically significant, it is interesting that the age of disease manifestation was 7.1 years (62.5, 95% CI 51.9 to 68.4, years *v* 55.4, 95% CI 49.1 to 62.4, years; $P = .38$) higher in *Arg/Arg* than in *Trp/Trp* subjects.

Finally, logistic regression analysis performed to determine the effect of the β_3-AR Trp64Arg polymorphism on CAD disclosed that the *Arg* allele had no significant effect (relative

Table 1. Clinical Characteristics of Cases and Controls

	Cases (n = 1,000)	Controls (n = 1,000)	*P*
Age (yr)	60.6 (55.1-67.1)	60.5 (54.5-66.5)	.477
Female (%)	24.1	24.1	
History (%)			
Diabetes mellitus	22.8	11.4	.001
Smoking	44.0	35.2	.001
Hypertension	55.2	35.9	.001
Hypercholesterolemia	52.7	30.3	.001
Age at CAD manifestation < 40 yr	6.8	—	
Age at CAD manifestation < 50 yr	27.9	—	
Myocardial infarction	66.7	—	
Acute	9.1		
History of	57.6		
No. of diseased vessels (% of patients)			
1 vessel	29.7	—	
2 vessels	36.5		
3 vessels	33.8		
BMI (kg/m^2)	26.3 (24.2-28.6)	26.0 (24.0-28.7)	.182
WHR	1.0 (0.9-1.0)	1.0 (0.9-1.0)	.659
Triglycerides (mmol/L)	1.7 (1.3-2.4)	1.5 (1.1-2.0)	.001
Cholesterol (mmol/L)	5.8 (5.1-6.6)	5.5 (4.7-6.3)	.001
HDL cholesterol (mmol/L)	1.1 (0.9-1.4)	1.1 (0.9-1.4)	.727
LDL cholesterol (mmol/L)	3.8 (3.1-4.5)	3.6 (2.9-4.2)	.001
apo-A1 (g/L)	1.4 (1.2-1.6)	1.4 (1.2-1.6)	.010
apo-B (g/L)	1.2 (1.0-1.4)	1.1 (0.9-1.3)	.001

NOTE. Data presented as medians and 25th to 75th percentiles.

risk 0.89; 95% CI 0.69 to 1.14; $P = .35$) on CAD, in contrast to established coronary risk factors (Table 2).

DISCUSSION

We investigated the relationship between the Trp64Arg polymorphism of the β_3-AR gene and CAD in a large sample of patients with and without angiographically assessed CAD. The rationale for this case-control study was that this hereditary trait has been reported to be linked to atherogenic risk factors,[1-3,5-7,15] including features of the insulin resistance syndrome.[30] Because the quantity of the *Arg/Arg* genotype studied until now was too small to support valid conclusions, the question remained whether and to what extent *Arg/Arg* influences the cardiovascular risk factor profile. Because the large sample size of 2,000 cases and controls, we were able to identify 328 *Trp/Arg* and 13 *Arg/Arg* subjects. The key finding of the present study was that there is no significant association between the β_3-AR polymorphism and the development and severity of CAD. Second, there was no significant influence of the *Trp/Arg* genotype and, at most, a weak impact of *Arg/Arg* on the cardiovascular risk factor profile and on the other clinical variables studied.

Table 2. Risk Factors for CAD in Logistic Regression Analysis Adjusted for Age and Sex

	Coefficient β	*P*	Relative Risk (95% CI)
Hypercholesterolemia	0.9183	<.000	2.5051 (2.07-3.03)
Diabetes mellitus	0.7897	<.000	2.2026 (1.69-2.86)
Hypertension	0.7477	<.000	2.1122 (1.74-2.57)
Smoking	0.5334	<.000	1.7048 (1.39-2.09)
Arg allele (carriers *v* noncarriers)	−0.1164	.3585	0.8901 (0.69-1.14)

Our study showed an *Arg* allelic frequency of 8.8% for cases and 9.0% for controls. These results agree with frequencies ranging between 6% and 13%, as published in recent studies of various white populations: primarily type 2 diabetic and grossly obese subjects.[1-3,5,8-10,15,16,18,21] We found no difference in frequency of *Trp/Arg* between patients with CAD and the control group. Apart from a slight trend, *Arg/Arg* subjects were not significantly overrepresented in the case group or in subgroups of cases, including females, hypertensives, and diabetics. Moreover, the *Arg* allele was not associated with early disease manifestation, myocardial infarction, or severity of CAD. In particular, logistic regression analysis excluded the possibility of significant effects of either the *Trp/Arg* or *Arg/Arg* genotype on the development and severity of CAD.

There is some evidence that the β_3-AR is associated with the presence of obesity-linked metabolic disturbances.[1-3,5-7,15] β_3-AR messenger RNA expression was recently detected in various human adipose tissue depots.[31] A subsequent study suggested that the β_3-AR is expressed mainly in the omental fat depot and contributes to catecholamine-induced lipolysis,[22] although others have reported little expression of this receptor subtype in human tissue.[32] An interesting observation is that sensitivity of the β_3-AR upon catecholamine stimulation is markedly increased in visceral obesity.[22] One might subsequently speculate that the resulting increase in free fatty acid release into the portal system may promote metabolic disturbances in the liver as well as in other organs.

To obtain a better understanding of the functional role of the β_3-AR polymorphism, it is necessary to examine the cellular level. Until now, only limited in vitro data on the effect of the

mutation on fat cell metabolism are available. In a study from Sweden, the lipolytic response of visceral white fat cells to noradrenaline and a selective β_3-agonist was comparable in control subjects and in *Trp/Arg* subjects.[33] In a group of Pima Indians who were homozygous *Arg/Arg*, subcutaneous adipose tissue lipolysis assessed by microdialysis was not significantly different from carriers of the wild type.[34] However, Pima Indians have a strong genetic propensity for obesity and have a very high allele frequency for the mutation: ie, approximately 10 times the rate of whites.[1] Moreover, only one functional parameter has been studied, in a fat depot known to contain only small amounts of β_3-AR.[22,31] For this reason, the question of whether the *Arg* allele has functional significance in humans is unsettled, and further studies are needed.

In our study population with 13 *Arg/Arg* subjects, we did not find substantial differences among the three genotypes in serum lipid concentrations. These variables included triglycerides; total, LDL, and HDL cholesterol; apo-A1; and apo-B. This finding is in accord with recently published literature[21] and argues against the clinically relevant impact of the β_3-AR polymorphism on lipid metabolism.

In contrast to results obtained in a recent study in a Japanese population,[19] *Arg/Arg* subjects in the case group did not have higher blood pressure than did heterozygotes.

Investigation of serum lipids and the other biochemical and anthropometric variables showed no differences between the *Trp/Arg* subjects and wild types. However, the few *Arg/Arg* subjects in the control group had significantly lower total cholesterol concentrations than did the *Trp/Arg* subjects and the wild types, although the total number of *Arg/Arg* subjects was too small to support a firm conclusion.

Our results practically rule out a significant impact of the *Trp/Arg* genotype on risk factors for CAD. These findings support critical comments on its physiological importance[8-12,16-18,21] and contradict previously reported data describing an association with atherogenic risk factors.[1-3,5-7,15,19]

In conclusion, the results of this study refute an association of the Trp64Arg polymorphism with the onset and severity of CAD. It was only in the small group of *Arg/Arg* subjects that we detected rather weak favorable impact on the metabolic risk factor profile: an argument against a significant role of the mutation in the development of CAD.

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