Haplotype Analysis of the $\beta 2$ Adrenergic Receptor Gene and Risk of Myocardial Infarction in Humans

Robert Y. L. Zee,^{*,1} Nancy R. Cook,^{*} Rebecca Reynolds,[†] Suzanne Cheng[‡] and Paul M. Ridker^{*}

^{*}Center for Cardiovascular Disease Prevention, LeDucq Center for Molecular and Genetic Epidemiology and Harvard-Reynolds Center for Cardiovascular Research, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02215, [†]Bayer HealthCare, East Walpole, Massachusetts 02032 and [‡]Department of Human Genetics, Roche Molecular Systems, Alameda, California 94501

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ABSTRACT

Polymorphisms in the $\beta 2$ adrenergic receptor (ADRB2), in particular G16R, Q27E, and T164I, have been implicated in the pathogenesis of cardiovascular and metabolic phenotypes. However, no prospective, genetic-epidemiological data are available on the risk of cardiovascular disease associated with these variants. Using DNA samples collected at baseline in a prospective cohort of 14,916 initially healthy American men, we evaluated the G16R, Q27E, and T164I polymorphisms among 523 individuals who subsequently developed myocardial infarction and among 2092 individuals who remained free of reported cardiovascular events during follow-up. The haplotype frequency distribution was significantly different among cases and controls ($\chi^2_{d.f.} = 20.92$, $P = 0.0039$). Haplotype-based logistic regression, adjusting for age, smoking, and randomized treatment group, indicated that G16-Q27-T164 (odds ratio 0.178, 95% C.I. 0.043–0.737, $P = 0.017$) and (non-G16-Q27)-T164 (odds ratio 1.235, 95% C.I. 1.031–1.480, $P = 0.022$) haplotypes were significantly associated with altered risk of myocardial infarction. These findings remained after further adjustment for BMI, history of hypertension, and presence or absence of diabetes. In conclusion, variation in haplotype frequencies for the $\beta 2$ adrenergic receptor gene was found to be associated with risk of myocardial infarction.

THE $\beta 2$ adrenergic receptor (ADRB2), a member of the G-protein-coupled receptor superfamily, and its associated signaling complex play an important role in the pathogenesis of cardiovascular and metabolic disorders (JOHNSON and TERRA 2002). A number of polymorphisms in *ADRB2* have been identified; in particular, G16R, Q27E, and T164I polymorphisms have been shown to have markedly differential effects on functional responses to adrenergic stimulation. Recently, these polymorphisms have been implicated in various cardiovascular and noncardiovascular disease phenotypes, including essential hypertension, asthma, obesity, and type 2 diabetes (DRYSDALE *et al.* 2000; HOFFSTEDT *et al.* 2001; HERRMANN *et al.* 2002; KAY *et al.* 2003). However, to date, no prospective, genetic-epidemiological data are available on the risk of athero-thrombotic disease. We therefore examined the role of G16R, Q27E, and T164I nonsynonymous polymorphisms, or haplotype patterns thereof, as risk determinants of incident myocardial infarction (MI) in a large, prospective cohort of apparently healthy men.

MATERIALS AND METHODS

Patient selection and clinical investigation: We used a nested case-control design within the Physicians' Health Study (STEERING COMMITTEE OF THE PHYSICIANS' HEALTH STUDY RESEARCH GROUP 1989). In brief, of 22,071 American men aged 40–84 years who were free of prior MI, stroke, transient ischemic attack, and cancer, 14,916 provided baseline blood samples, which were available for genetic analysis. In our overall nested case-control approach, each participant who provided an adequate sample of whole blood at baseline and had a confirmed MI, ischemic stroke, or venous thrombosis during follow-up was matched wherever possible to two or three controls. The controls were study participants who had also provided a baseline blood sample and who remained free of any reported cardiovascular disease at the time the index event occurred in the case patient. Controls were selected at random from among those who met the matching criteria of age (± 2 years), smoking habits, and time since study entry. As previously described (STEERING COMMITTEE OF THE PHYSICIANS' HEALTH STUDY RESEARCH GROUP 1989), yearly follow-up self-report questionnaires provide reliable updated information on newly developed diseases and the presence or absence of other cardiovascular risk factors. For all reported incident vascular events occurring after study enrollment, hospital records, death certificates, and autopsy reports were requested and reviewed by an end points committee using standardized diagnostic criteria. The diagnosis of MI was confirmed by evidence of symptoms in the presence of either diagnostic elevations of cardiac enzymes or diagnostic changes on electrocardiograms. In the case of fatal events, the diagnosis of MI was also accepted on the basis of autopsy findings. To increase

¹Corresponding author: Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Harvard Medical School, 900 Commonwealth Ave. East, Boston, MA 02215.
E-mail: rzee@rics.bwh.harvard.edu

TABLE 1

Baseline characteristics of study participants who subsequently developed myocardial infarction (cases) and those who remained free of vascular disease during follow-up (controls)

	Cases ^a (N = 523)	Controls ^a (N = 2092)	P
Age (yr)	58.7 ± 0.4	58.8 ± 0.2	0.83
Smoking status (%)			0.96
Never	42.9	43.5	
Past	42.0	41.3	
Current	15.1	15.3	
Body mass index (kg/m ²)	25.5 ± 0.1	25.0 ± 0.1	<0.001
Blood pressure (mm Hg)			
Systolic	129.8 ± 0.6	127.4 ± 0.3	<0.001
Diastolic	80.6 ± 0.4	79.0 ± 0.2	<0.001
Hyperlipidemia (%)	13.2	8.3	<0.001
Diabetes (%)	5.6	2.7	0.001
Family history of premature CAD (%)	13.3	8.0	<0.001

^a Mean ± SE.

power for statistical analysis, all controls were pooled in a common reference group. A total of 523 cases of incident MI and 2092 controls were available for the current analysis. The study was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

Genotyping: Genotype determination for the ADRB2 polymorphisms was accomplished using multiplex PCR and immobilized probe-based assays developed for multi-locus variant detection (Roche Molecular Systems, Alameda, CA) essentially as described elsewhere (CHENG *et al.* 1999; ZEE *et al.* 2002). Genotype scoring was carried out by two independent observers. Discordant results (<2%) were resolved by a further joint reading and, where necessary, by a repeat genotyping. All results were scored blinded to case-control status. Because of the rarity of the I164 allele observed in the case group, a second method, polymerase chain reaction-restriction fragment length polymorphism as described by KAY *et al.* (2003), was used to regenotype the entire case group, and we found 100% concordance with our original genotyping score.

Statistical analysis: Demographic and clinical data between cases and controls were compared using χ^2 analysis (categorical variables) and *t*-test (continuous variables). Genotype frequencies between cases and controls were compared by χ^2 analysis. Pairwise linkage disequilibrium (LD) was examined using the software Haploview (<http://www.broad.mit.edu/personal/jcbarret/haploview/>). Haplotype frequencies were estimated from genotype data using the partition ligation-expectation maximization algorithm (QIN *et al.* 2002). Haplotype distributions were compared between cases and controls by a likelihood-ratio test (TERWILLIGER and OTT 1994). A likelihood-ratio statistic was computed from the estimated haplotype frequency likelihoods for cases and controls separately *vs.* combined. In addition, the relationship between the haplotypes and risk of MI was examined by a haplotype-based logistic

TABLE 2

Genotype and allele distribution among study participants who subsequently developed myocardial infarction (cases) and those who remained free of vascular disease during follow-up (controls)

Polymorphism	Cases (%)	Controls (%)	$\chi^2_{2d.f.}$ ^a	P
G16R				
G16G16	194 (37.90)	829 (39.63)	1.54	0.46
G16R16	240 (45.89)	943 (45.08)		
R16R16	89 (17.02)	320 (15.30)		
G16	0.60	0.62		
R16	0.40	0.38		
Q27E				
Q27Q27	179 (34.23)	745 (35.61)	1.40	0.50
Q27E27	240 (45.89)	977 (46.70)		
E27E27	104 (19.89)	370 (17.69)		
Q27	0.57	0.59		
E27	0.43	0.41		
T164I				
T164T164	521 (99.62)	2039 (97.47)	9.40 ^b	0.0022
T164I164	2 (0.38)	53 (2.53)		
I164I164	0	0		
T164	0.998	0.987		
I164	0.002	0.013		

^a χ^2 test for genotype distribution.

^b 1 d.f.

regression analysis with baseline-haplotype parameterization (WALLENSTEIN *et al.* 1998), adjusting for age, body mass index (BMI), hypertension, smoking habits, diabetes, and randomized aspirin/ β -carotene treatment assignment. This parameterization approach, using an additivity assumption, is particularly appropriate for case-control studies. A global test statistic, comparing the model with genetic data to the model without genetic data using the likelihood-ratio test, was also performed to check for the overall association of haplotypes with the disease outcome. For each odds ratio, we calculated 95% confidence intervals (C.I.'s). A two-tailed *P*-value of 0.05 was considered a statistically significant result.

RESULTS

Baseline characteristics of the study population are shown in Table 1. As expected in a prospective cohort study, participants who subsequently developed MI had a higher prevalence of conventional athero-sclerotic risk factors at baseline than did the control subjects. The observed genotype distributions were in Hardy-Weinberg equilibrium among the controls. Using a standard marker-by-marker χ^2 analysis, genotype distribution was significantly different between cases and controls only for T164I (*P* = 0.0022; Table 2). The polymorphisms tested were in strong LD among one another (Table 3). Further analysis using haplotypic information showed a significant difference in haplotype distribution between cases and controls (*P* = 0.004; Table 4). As shown in Table 4, three of the eight possible haplotypes were not

TABLE 3

Pairwise linkage disequilibrium analysis

D', r ²	G16R	χ ² , P: Q27E	T164I
G16R	—	2282.4, <0.0001	34.5, <0.0001
Q27E	1.0; 0.436	—	39.3, 0.30
T164I	1.0; 0.006	1.0; 0.008	—

D', Lewontin's normalized value.

observed in our study sample (G16-E27-I164, R16-Q27-I164, R16-E27-I164), and the R16-E27-T164 haplotype was extremely rare and not present in the control group; these haplotypes were not included in the haplotype-based logistic regression analysis. As also shown in Table 4, virtually all of the I164 carriers were G16-Q27 whereas a minority of the T164 carriers were G16-Q27, a result of an apparent linkage disequilibrium between the G16-Q27 haplotype and the T164 allele. Thus, the haplotype patterns were further collapsed and stratified using T164 *vs.* I164 and G16-Q27 *vs.* non-G16-Q27 as presented in Table 5. Further analysis of the combined-genotype distribution after excluding the T164I heterozygotes yielded similar significant results ($P < 0.0001$; APPENDIX). Thus, G16-Q27-T164 was used as the baseline haplotype for the regression analysis. Results from the crude logistic regression analysis showed significant association between haplotype patterns G16-Q27-I164 and (non-G16-Q27)-T164 with risk of MI ($P = 0.017$ and $P = 0.022$, respectively; Table 6). Further adjustment for BMI, hypertension status, and diabetes yielded similar findings ($P = 0.022$ and $P = 0.015$, respectively; Table 6). The global test statistic—comparing the model with genetic data to the model without—again showed similar significant results (crude, $P < 0.001$; adjusted, $P < 0.001$; Table 6). The haplotype-based logistic regression approach proposed by WALLENSTEIN *et al.* (1998) to analyze the data in the present study was made possible by the particular LD pattern among the three polymorphisms investigated, which allowed unambiguous assignment of haplotype pairs to each individual (confidence level for the haplotype estimation and inference >99.90%). Virtually identical study results were obtained in an analysis limited to the Caucasian male participants (data not shown).

DISCUSSION

We investigated the association of three nonsynonymous *ADRB2* polymorphisms and the risk of MI. We found no evidence for a single-marker association between G16R or Q27E and the risk of MI; however, we found a significant protective effect of the T164I variant with MI. As previously reported, strong linkage disequilibrium was observed among the three polymorphisms. Further analysis using haplotype frequencies indicated

TABLE 4

Estimated haplotype frequency among study participants who subsequently developed myocardial infarction (cases) and those who remained free of vascular disease during follow-up (controls)

Haplotype	Cases	Controls
G16-Q27-T164	0.171	0.198
G16-Q27-I164	0.002	0.013
G16-E27-T164	0.427	0.410
G16-E27-I164	0.000	0.000
R16-Q27-T164	0.399	0.378
R16-Q27-I164	0.000	0.000
R16-E27-T164	0.001	0.000
R16-E27-I164	0.000	0.000

χ²_{d.f.} = 20.92; P -value for likelihood-ratio test = 0.00389.

that haplotypes G16-Q27-I164 and (non-G16-Q27)-T164, as compared to the reference haplotype G16-Q27-T164, were significantly associated with an altered risk of MI, suggesting a role for the G16-Q27 combination in addition to the individual protective effect of T164I polymorphism.

The I164 allele has recently been shown to display a threefold reduction in receptor affinity, a markedly depressed basal and agonist-stimulated adenylyl cyclase activity, an impaired stabilization of mast cells, and a decrease in adipocyte sensitivity as compared to the T164 allele (DRYSDALE *et al.* 2000; HOFFSTEDT *et al.* 2001; HERRMANN *et al.* 2002; JOHNSON and TERRA 2002; KAY *et al.* 2003). Moreover, the I164 allelic form of the receptor has been associated with increased downregulation as compared to the T164 form (GREEN *et al.* 1993). Interestingly, a recent report by LIGGETT *et al.* (1998), examining the role of G16R, Q27E, and T164I polymorphisms in heart failure, has shown an association of the T164I polymorphism with the outcome of patients with congestive heart failure; individuals with heart failure carrying the I164 allele were at significant risk for rapid progression as compared to those carrying the T164 allele. However, this finding was based on only 10 pa-

TABLE 5

Prespecified estimated haplotype frequency among study participants who subsequently developed myocardial infarction (cases) and those who remained free of vascular disease during follow-up (controls)

Haplotype	Cases	Controls	χ ² _{d.f.}	P ^a
G16-Q27-T164	0.171	0.198	14.15	<0.001
G16-Q27-I164	0.002	0.013		
(non-G16-Q27)-T164	0.827	0.788		
(non-G16-Q27)-I164	0.000	0.000		

Non-G16-Q27 is G16-E27, R16-Q27, and R16-E27 combined.

^a P -value for likelihood-ratio test.

TABLE 6

Haplotype-based logistic regression analysis for risk of myocardial infarction and baseline-haplotype parameterization with G16-Q27-T164 as the reference haplotype

	Odds ratio	95% C.I.	<i>P</i>	$\chi^2_{2d.f.}$ ^a	<i>P</i> ^a
Crude				18.238	<0.001
G16-Q27-T164	0.178	0.043–0.737	0.017		
(non-G16-Q27)-T164	1.235	1.031–1.480	0.022		
Adjusted				17.893	<0.001
G16-Q27-T164	0.189	0.045–0.785	0.022		
(non-G16-Q27)-T164	1.255	1.045–1.508	0.015		

Crude, controlling for age, smoking, and randomized treatment group. Adjusted, further controlling for BMI, history of hypertension, and presence or absence of diabetes. non-G16-Q27 is G16-E27, R16-Q27, and R16-E27 combined.

^a χ^2 and *P*-values for global likelihood-ratio test comparing model with haplotypes to model without.

tients with the T164 allelic form and should be interpreted with caution (JOHNSON and TERRA 2002).

By comparison, the G16R and Q27E polymorphisms have not been shown to have an effect on either ligand binding or adenylyl cyclase activity. However, they have been reported to have influence on receptor desensitization (JOHNSON and TERRA 2002). A recent study by D'AMATO *et al.* (1998) examining the association of G16R and Q27E with bronchial hyperresponsiveness (BHR) using haplotypic information found an increased frequency of the G16-Q27 haplotype among BHR-positive subjects, suggesting that this haplotype may be an independent risk marker for BHR. In experimental studies, the G16 and Q27 alleles have been independently associated with increased downregulation (GREEN *et al.* 1994, 1995), further suggesting that this haplotype carrying these allelic forms has the greatest potential for receptor downregulation. Interestingly, in our investigation, a discrepancy was observed between the pre-specified combined-genotype analysis (APPENDIX) and the haplotype-based analysis (Table 5) on G16R and Q27E with risk of MI. The G16R16-Q27Q27 combined genotype is common among the cases, but has a relatively low frequency among the controls. On the other hand, the G16R16-Q27E27 combined genotype is common among the controls, but is relatively infrequent among the cases (APPENDIX), suggesting that having one R16 is associated with increased risk, while having one R16 and one E27 is associated with reduced risk. The discrepancy might result from the nature of the additivity assumption that underlies the haplotype-based logistic regression approach used and/or suggest additional complexity of heterogeneous interaction between G16R and Q27E.

Taken together, our observed associations of the T164I polymorphism, either alone or in a haplotype context, with reduced risk of MI provide further evidence of roles for β_2 adrenergic receptor gene polymorphisms in athero-thrombotic disease, although the exact mech-

anism(s) by which the gene variants/haplotypes that were tested influence disease progression remain unclear. We cannot exclude the possibility that the polymorphisms/haplotypes examined are in linkage disequilibrium with an unidentified susceptibility gene(s)/polymorphism(s) that is responsible for the observed significant associations and for the additional complexity of heterogeneous interaction observed between G16R and Q27E. These results do, however, suggest the importance of including a haplotype-based analysis, as others have recommended (SCHMIDT 2002; SCHMIDT *et al.* 2002), for proper assessment of multi-locus genetic association studies (JOHNSON and TERRA 2002).

The Physician's Health Study cohort is almost exclusively composed of Caucasian middle-aged men, and therefore the present data may not be generalized to other ethnic groups, women, or other populations. Nonetheless, there are considerable strengths of our present study design. It is a large prospective, nested case-control cohort in which the determination of case status is based solely on the subsequent development of disease rather than on arbitrary selection criteria. The background allele frequencies observed in our control group were similar to those previously reported in other populations (D'AMATO *et al.* 1998; LIGGETT *et al.* 1998; HOFFSTEDT *et al.* 2001; JOHNSON and TERRA 2002; KAY *et al.* 2003).

In conclusion, on the basis of the studied population, we found an association of the β_2 adrenergic receptor gene variants with altered risk of myocardial infarction. Furthermore, if replicated in other studies, our findings provide further evidence of an important role for the ADRB2 gene and its variants in athero-thrombotic disorders.

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APPENDIX

Prespecified combined-genotype frequency between G16R and Q27E pairs among study participants who subsequently developed myocardial infarction (cases) and those who remained free of vascular disease during follow-up (controls)

Combined genotype ^a	Cases	Controls
G16G16-Q27Q27	0.029	0.042
G16G16-Q27E27	0.140	0.183
G16G16-E27E27	0.200	0.181
G16R16-Q27Q27	0.311	0.166
G16R16-Q27E27	0.150	0.270
R16R16-Q27Q27	0.171	0.157

^a Only T164T164 homozygotes were considered. $\chi^2_{5df} = 76.40$; $P = <0.0001$.