Variation in bradykinin receptor genes increases the cardiovascular risk associated with hypertension

Sukhbir S. Dhamrait, John R. Payne, Ping Li, Alun Jones, Iqbal S. Toor, Jacqueline A. Cooper, Emma Hawe, Jutta M. Palmen, Peter T. E. Wootton, George J. Miller, Steve E. Humphries, Hugh E. Montgomery

Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, Royal Free & University College London Medical School, Rayne Building, London, UK

Medical Research Council Cardiovascular Research Group, Wolfson Institute of Preventive Medicine, London, UK

Received 14 March 2003; received in revised form 26 June 2003; accepted 9 July 2003

Aims The contribution of kinins to the beneficial effects of angiotensin I converting enzyme (ACE) inhibition in cardiovascular risk reduction remains unclear. The genes for the kinin inducible B1 receptor (B1R) and constitutive B2 receptor (B2R) contain functional variants: the B1R−699C (rather than G) and the B2R(−9) (rather than +9) alleles are associated with greater mRNA expression and the B2R(−9) allele with reduced left ventricular hypertrophic responses. We tested whether these gene variants influenced hypertensive coronary risk in a large prospective study.

Methods and results Two thousand, seven hundred and six previously healthy UK men (mean age at recruitment 56 years; median follow-up 10.8 years) were genotyped for the kinin receptor variants. The coronary risk attributable to systolic hypertension (SBP≥160 mmHg) was significantly higher only in B1R−699GG homozygotes (HR 2.14 [1.42–3.22]; P<0.0001) and B2R(+9,+9) individuals (HR 3.51 [1.69–7.28]; P=0.001) but not in B1R−699C allele carriers (HR 0.82 [0.28–2.42]; P=0.76) or in B2R(−9,−9) homozygotes (HR 1.25 [0.51–3.04]; P=0.63).

Conclusions Common variation in the genes for the kinin B1 and B2 receptors influences prospective hypertensive coronary risk. These are the first reported human data to suggest a role for the B1R in human coronary vascular disease, and the first prospective study to demonstrate a similar role for the B2R.

© 2003 Published by Elsevier Ltd on behalf of The European Society of Cardiology.

KEYWORDS
Bradykinin; receptor; polymorphism; hypertension; risk; coronary disease

Introduction

The reduction in vascular mortality and morbidity associated with pharmacological angiotensin I converting enzyme (ACE) inhibition is generally attributed to a reduction in local vascular angiotensin II (Ang II) genesis, given the latter’s potent effects on metabolic, coagulation, and inflammatory pathways. However, although treatment with Ang II type 1 receptor (AT1R) blockers (ARBs) may reduce cardiovascular risk in part through non-hypotensive mechanisms, ARBs have yet to be proven equieffective with ACE inhibitors in reducing cardiovascular risk. Attention has thus shifted to the role of ACE as a pivotal component of the vascular kallikrein-kinin system (KKS), where it is responsible for the degradation of the vasodilator/vasculoprotective nonapeptide bradykinin. Indeed bradykinin, rather than angiotensin I, seems the preferred substrate of ACE.

Kinins are endogenous ligands for G-protein coupled inducible B1 and constitutive B2 receptors (B1R and B2R respectively), whose activation may play a role in the modulation of atherosclerotic risk through promotion of
microangiogenesis,7 inhibition of vascular smooth muscle cell growth,8 coronary vasodilatation,9 increased local nitric oxide synthesis,10 and anti-thrombotic actions.11 Reduced kinin degradation may thus contribute to the beneficial effects of ACE inhibition on vascular risk.12 However, proof is lacking. Genetic studies can help to address this issue.

The kinin B₂R and B₁R genes have been mapped to human chromosomal region 14q32.1-q32.2. A common functional variant has recently been described in exon 1 of the gene for the B₂R, in which the presence (+9) rather than the absence (−9) of a nine-base pair repeat sequence is associated with lower gene transcriptional activity13 and lower mRNA expression.14 Similarly a G>C variant at position −699 in the promoter region of the B₂R gene exists, in which the C allele is associated with higher promoter activity.15

If raised kinin activity is indeed vasculoprotective, we would anticipate that low kinin receptor activity, as marked by the B₂R(+9) and B₂R−699G alleles, would be associated with excess prospective cardiovascular risk. In addition, hypertensive left ventricular (LV) hypertrophy is an independent cardiovascular risk factor,16 and reduced kinin receptor activity is associated with LV hypertrophic responses in animals17 and humans.18 Thus, reduced kinin receptor activity may bridge hypertrophic responses and risk of coronary artery disease (CAD). As such, we might postulate that genotype would especially influence risk amongst hypertensive individuals. We have examined these hypotheses in a large prospective study of healthy UK men.

Methods

Ethical approval was granted by the institutional ethical committee and all subjects gave written informed consent.

Study sample

Subjects comprised those recruited to the Second Northwick Park Heart Study (NPHSII), described in detail elsewhere.19 In brief, 2706 unrelated healthy Caucasian middle-aged male subjects (mean age 56.1±3.5 years) recruited from nine UK general practices and prospectively followed for a median of 10.8 years (range 7 days to 13.2 years) were eligible for analysis. Eligibility was conditional on the subject not having myocardial infarction (MI), cerebrovascular disease, life threatening malignancy or regular medication with aspirin or anticoagulants. Subjects receiving antihypertensive medication with normal blood pressure at entry into the trial were also excluded from analysis. At entry, systolic and diastolic (Korotkoff V) blood pressures (SBP and DBP respectively) were recorded twice with a random zero mercury sphygmomanometer after the subject had been seated for 5 min. The mean values were used in the statistical analysis. At trial inception, systolic hypertension was defined as SBP≥160 mmHg and diastolic hypertension as DBP≥95 mmHg,20 and was reported to the subject’s general practitioner for action. Baseline demographics and conventional risk factors for CAD were documented. Patients were monitored with annual examinations and regular review of medical records. Those who moved from their recruitment location were similarly followed up. Deaths were recorded through the UK National Health Service Central Register. Information for events presenting clinically/symptomatically were assembled by systematic enquiries through the participating practices, hospitals attended and for fatal events, coroners’ offices. CAD events were defined as sudden cardiac death, symptomatic MI (based on history, ECG, cardiac enzymes and pathology that were assessed by an independent reviewer who classified events by criteria of the World Health Organization21), silent MI (the appearance of a new major Q wave on the follow up ECG, using Minnesota codes 1,2,3,4,5,6,7,8,9,10,11,12,13,14 plus 5, or 5,22), or coronary revascularization (either surgical or percutaneous). In addition, any (rare) subclinical events were identified through routine electrocardiography at baseline and the sixth annual examination. Time to first coronary event was recorded, yielding only one event per subject. The likelihood of detecting any component endpoint event is thus considered equal across all sites. There have been 175 events comprising 124 (70.9%) acute MI, 33 (18.9%) coronary surgery and 18 (10.3%) silent MI.

Genotyping for B₁R−699G>C and B₂R(+9,−9) polymorphisms

At entry, a 5 ml EDTA blood sample was drawn, from which genomic leukocyte DNA was extracted by salting out. Genotypes were determined using polymerase chain reaction amplification (PCR) using published primers and conditions15 for the B₁R−699G>C and forward 5′-TCTGGCTTCTGGGCTCCGAG-3′ and reverse 5′-ACGGGATGCGAACCTTCACTG-3′ primers for the B₂R(+9,−9) polymorphisms, products resolved on a 7.5% polyacrylamide gel and confirmed by two independent technicians blind to subject outcome, with discrepancies resolved by repeat genotyping.

Statistical analysis

Analysis was performed using ‘Intercooled STATA’ software (version 7.0, STATA Corporation, Texas). Data are reported for those individuals amongst whom high-throughput genotyping was successful. Deviations from Hardy–Weinberg were considered using chi-squared tests and allelic association between the genotypes was considered via the correlation coefficient δ. Log-transformations were conducted for data which were not normally distributed. One-way analysis of variance was used to assess the effect of genotypes on baseline characteristics, using either the raw values or log-transformed values, as appropriate. There was no evidence of heteroscedacity between groups, considered via Bartlett’s test. Survival analysis with respect to genotypes was carried out using Cox’s proportional hazards model, thus allowing for varying follow-up intervals and censoring due to competing events. For this modelling, ‘failure time’ was taken as the time to the first CAD event. The significance of the parameters in the Cox model was assessed using the likelihood ratio (LR) Test. 95% confidence intervals (CI) for the estimates were calculated from the standard errors assuming a normal distribution. All results were exponentiated and are presented as hazard ratios (HR) with their corresponding 95% CI. Comparisons of interest were hazard ratios for systolic and diastolic hypertension, genotype, and for a 1 standard deviation increase in diastolic and systolic blood pressure. Survival analysis was adjusted for age by including the term in the model and differences in the baseline hazard by practice were permitted (using the strata option in STATA). To adjust for established CHD risk factors, body mass index (BMI), smoking, diabetes, cholesterol and triglyceride were also included as covariates in the model. Interactions were considered as deviations from multiplicative effects in the survival model. Hazard ratios within genotype subgroups were obtained from models including a
Results

Baseline characteristics by CAD event status, genotype distribution and allele frequencies for all subjects from the Second Northwick Park Heart Study (NPHSII) genotyped for either the kinin B1R−699G>C or B2R(+9,−9) gene variants. Data are mean (SD) unless otherwise stated.

Table 1. There was no difference in baseline characteristics in those genotyped and those not successfully genotyped for either polymorphic variant (data not shown). Due to the rarity of CC homozygotes, the C allele carriers were grouped together for further statistical analysis, and analysis corrected for smoking. There was no significant difference in C allele frequency amongst subjects with or without systolic hypertension (C allele frequency 0.09 and 0.11 respectively; P=0.58), and there was no difference by genotype in the development of hypertension during follow-up in those normotensive at baseline.

Coronary event probability in relation to increasing SBP in subjects divided by B1R−699 genotype is depicted in Fig. 1b. Risk increased significantly with increasing blood pressure only in GG homozygotes, but not in C allele carriers (hazard ratio for a 1 standard deviation increase in SBP 1.34 [1.14–1.58]; P=0.001 and 1.19 [0.84–1.70]; P=0.34 respectively). In the overall study group, there was no association between B1R genotype and cardiovascular risk (HR 1.0 [0.66–1.51]; P=0.99 for GG vs C allele carriers). The risk associated with systolic hypertension (SBP≥160 mmHg) was significant in GG homozygotes (HR 2.14 [1.42–3.22]; P=0.0001) but not in C allele carriers (HR 0.82 [0.28–2.42]; P=0.76; Table 2; P for interaction=0.11). Risk remained statistically significant after adjustment for other established CAD risk factors (risk adjusted for age, practice, BMI, smoking, diabetes, cholesterol and triglycerides; Table 2). Risk estimates were essentially unchanged when only acute (fatal and non-fatal) MI events were considered.

Similar effects were seen when assessing the increase in risk associated with diastolic hypertension (GG homozygotes: HR 2.65 [1.79–3.91]; P=0.0001, C allele carriers: HR 1.23 [0.49–3.08]; P=0.66 compared to normotensives for each group).

B1R polymorphism

Of those eligible, genotype was obtained in 2252 subjects (83.2%) for the B1R−699G>C polymorphism. The genotype distribution was consistent with Hardy–Weinberg equilibrium (χ²=0.17; P=0.68) and both genotype distribution and rare allele frequency (0.09) were similar to those previously reported. There was a higher frequency of current smoking amongst CC homozygotes (current smoking GG 28.7%, GC 34.0% and CC 44.4%; P=0.04), but all other baseline characteristics were independent of genotype. In particular, there was no difference in either SBP or DBP by genotype (P=0.88, P=0.64 respectively). Due to the rarity of CC homozygotes, the C allele carriers were grouped together for further statistical analysis, and analysis corrected for smoking. There was no significant difference in C allele frequency amongst subjects with or without systolic hypertension (C allele frequency 0.09 and 0.11 respectively; P=0.58), and there was no difference by genotype in the development of hypertension during follow-up in those normotensive at baseline.

Table 1. Baseline characteristics and genotype frequencies by coronary artery disease event status for 2541 men from the Second Northwick Park Heart Study (NPHSII) genotyped for either the kinin B2R gene variants. Data are mean (SD) unless otherwise stated.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Controls No CAD event</th>
<th>Cases CAD event</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.0 (3.4)</td>
<td>56.5 (3.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>137.7 (19.1)</td>
<td>143.1 (20.7)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.1 (11.4)</td>
<td>87.6 (12.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>26.4 (3.5)</td>
<td>27.1 (3.5)</td>
<td>0.007</td>
</tr>
<tr>
<td>Current smoking % (n)</td>
<td>28.3% (670)</td>
<td>37.7% (66)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes % (n)</td>
<td>2.0% (47)</td>
<td>6.3% (11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (mmol l⁻¹)</td>
<td>5.71 (1.00)</td>
<td>6.09 (1.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride (mmol l⁻¹)</td>
<td>1.75 (0.92)</td>
<td>2.13 (1.15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B1R−699G&gt;C variant GG/GC/CC</td>
<td>1724/358/17</td>
<td>125/27/1</td>
<td>0.94</td>
</tr>
<tr>
<td>B1R variant (+9,+9)/(+9,−9)/(−9,−9)</td>
<td>572/1088/535</td>
<td>35/95/39</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*For triglyceride, the mean is geometric (approximate SD). CAD indicates coronary artery disease; B1R, bradykinin B1 receptor; B2R, bradykinin B2 receptor.
Fig. 1  Cardiovascular event probability modelled over a median 10.8 years of follow up in relation to systolic blood pressure in: (a) 2541 subjects with genotypic data, demonstrating the expected strong relationship between elevated blood pressure and CAD event rate (95% confidence intervals are shown as dotted lines); (b) subjects divided by B1R−699G>C genotypes; increasing risk amongst C allele carriers only; (c) subjects divided by B2R(+9,−9) homozygosity; increasing risk amongst (+9,−9) only.
B₂R polymorphism

B₂R genotype was obtained for 2364 (87.4%) participants and genotype frequencies were as expected for a population in Hardy–Weinberg equilibrium ($\chi^2=0.003; P=0.96$). The (−9) allele frequency was 0.49 and similar to previous published reports for a white Caucasian population. Baseline characteristics did not vary by B₂R genotype. In particular there was no association with DBP or SBP. The (−9) allele frequency was 0.50 and 0.48 amongst normotensives and subjects with systolic hypertension in (+9,+9) and (+9,−9) respectively. There was no significant association between B₂R genotype and cardiovascular events in the study overall. Amongst normotensives and subjects with systolic hypertension over the follow up period.

Coronary event probability in relation to increasing SBP in subjects divided by homozygosity for the B₂R variant is depicted in Fig. 1c. Risk increased significantly with increasing blood pressure in (+9,+9) and (+9,−9) groups, but there was no significant increase risk amongst (−9,−9) carriers (hazard ratio for a 1 standard deviation increase in SBP $1.58 \pm 2.11$; $P=0.002$, 1.33 [1.09–1.61]; $P=0.004$ and 1.12 [0.82–1.52]; $P=0.47$ for (+9,+9), (+9,−9) and (−9,−9) respectively). There was no significant association between B₂R genotype and cardiovascular events in the study overall. Amongst normotensives, compared to the (+9,+9) men, the CAD event rate tended to be higher in those carrying one or more (−9) alleles (HR 1.67 [1.05–2.64]; $P=0.03$) and this effect was statistically significant in the larger (+9,−9) heterozygote group normotensive at baseline (HR (+9,+9): 1.0; (+9,−9): 1.70 [1.05–2.74]; $P=0.03$). However, as shown in Table 2, hypertension significantly increased the cardiovascular risk in B₂R(+9,+9) individuals when compared to their normotensive counterparts (HR 3.51 [1.69–7.28]; $P=0.001$ and HR 2.65 [1.31–5.38]; $P=0.007$ for systolic and diastolic hypertension respectively), but not in B₂R(−9,−9) homozygotes (HR 1.25 [0.51–3.04]; $P=0.63$ and HR 1.23 [0.62–2.44]; $P=0.55$ for systolic and diastolic hypertension respectively, with $P=0.21$ for the interaction of B₂R genotype, SBP and risk). Risk estimates for homozogotes remained significant after adjustment for other CAD risk factors (Table 2) and when CAD events were confined to acute (fatal and non-fatal) MI events.

In order to examine whether the effect of the two genotypes on risk was additive, men were divided into groups on the basis of the combined B₁R and B₂R genotypes. The number of subjects within the B₂R−699C allele group was too small for meaningful analysis of interaction with B₂R genotype. However, when the CAD event rate was compared for those of B₁R−699GG genotype who were also homozygous for the B₂R(−9) allele, risk in the hypertensive group was substantially elevated (HR 4.92 [2.04–11.83]; $P=0.0001$). There was no such increase in risk in those who were homozygous for the B₂R−699G and B₂R(−9) alleles (HR 0.68 [0.22–2.65]; $P=0.68$) (Table 3).

The data are presented graphically as a Kaplan–Meier survival plot, demonstrating the considerably higher event rate in B₂R−699GG: B₂R(+9,+9) hypertensives (Fig. 2).

Discussion

Cardiovascular risk associated with hypertension amongst middle-aged men is influenced by functional variation in the genes for kinin B₁ and B₂ receptors: in the presence of the B₁R(+9) or B₂R−699G alleles, cardiovascular risk climbs steeply as blood pressure rises – an effect not identified amongst those homozygous for the B₂R(−9) or B₂R−699C alleles. The impact of genotype is exemplified by the substantial elevation of risk amongst those suffering systolic hypertension ($\geq 160$ mmHg, as dichotomously defined at trial inception some 14 years ago). Even when modern definitions are applied (e.g. SBP$\geq 140$ mmHg) the impact of genotype on risk remains statistically significant (data not shown). These findings persist after multivariate adjustment for all potential confounders, and genotype is unrelated to the presence or development of hypertension itself, in keeping with past observations. These are the first reported human data to suggest a role for the B₂R in human coronary
Table 3  Haplotype analysis of coronary artery disease (CAD) events in subjects stratified by presence of systolic hypertension (SBP≥160 mmHg). Subjects who are B1R−699GG homozygotes have been divided by homozygosity for B2R(+9,−9) polymorphism. Relative hypertensive risk is CAD risk of hypertensive vs normotensive subjects within each respective genotype group.

<table>
<thead>
<tr>
<th>B2R Genotype of B1R GG homozygotes</th>
<th>Normotensives SBP&lt;160 mmHg Events/Total No. (Events per 1000 patient years)</th>
<th>Hypertensives SBP&gt;160 mmHg Events/Total No. (Events per 1000 patient years)</th>
<th>Relative hypertensive risk [95% CI]</th>
<th>Probability</th>
<th>Adjusted relative hypertensive risk [95% CI]</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+9,+9)</td>
<td>12/362 (3.6)</td>
<td>11/74 (17.2)</td>
<td>4.92 [2.04–11.83]</td>
<td>&lt;0.0001</td>
<td>4.48 [1.71–11.74]</td>
<td>0.002</td>
</tr>
<tr>
<td>(−9,−9)</td>
<td>22/342 (7.0)</td>
<td>3/53 (6.1)</td>
<td>0.77 [0.22–2.65]</td>
<td>0.68</td>
<td>0.77 [0.21–2.78]</td>
<td>0.69</td>
</tr>
</tbody>
</table>

*Risk adjusted for age, practice and practice for B1R genotypes and age and practice for B2R genotypes.

**Risk adjusted for age, practice, smoking, diabetes, body mass index, cholesterol, triglycerides. SBP indicates systolic blood pressure; B1R, bradykinin B1 receptor; B2R, bradykinin B2 receptor.
vascular disease, and the first prospective study to demonstrate a similar role for the B2R. These data support a modulatory role for the human KKS in the pathogenesis of such disease.

Altered activity in the human coronary vascular KKS may underlie these findings. Kallikreins catalyse the conversion of kininogens to autacoid peptide kinins such as bradykinin and Lys-b Bradykinin, whose subsequent cleavage of the C-terminal arginine residue by carboxypeptidase yields the fragments des-Arg⁹-bradykinin and Lys-des-Arg⁹-bradykinin. Intact kinins and their fragments act upon the B₁ and B₂ G-protein coupled cell-surface receptors, which share only 36% sequence homology and differ greatly in their expression and pharmacology. Thus, the constitutive B₂R is expressed in diverse cell types including those of the endothelium, and is responsive to intact kinin peptides. Conversely, the inducible B₁R is activated by C-terminal arginine-deficient kinin fragments. The expression and function of these receptors is known to be altered by the polymorphisms used in this study. The B₁R−699G>C polymorphism lies within a positive control element and the C allele is associated with greater in vitro reporter gene transcription. The B₂R(−9) variant of the B₂R is associated with greater B₂R gene promoter activity and mRNA expression. These functional effects may underlie the reported associations with clinical disease states: the B₁R−699C allele is underrepresented amongst patients with end stage renal failure, whilst the B₂R(−9) allele is associated with symptomatic hereditary angioedema in cases of C1 inhibitor deficiency and with lower cardiac physiological hypertrophic responses. The B₁R and B₂R lie adjacent to one another on chromosome 14. The described variants may be in allelic association with other unidentified, functional variants within this chromosomal locus. However, the reported associations of the polymorphisms with differences in gene transcription and promoter activity suggest that the polymorphisms themselves have functional effects, or that they are in close linkage disequilibrium with adjacent intra-allelic functional sites. In addition, no function as yet has been assigned to different adjacent genes. However, the functional associations of the polymorphisms, and the fact that we, and others, have found no significant linkage disequilibrium across the region, mitigates against the observed phenotypic effects being mediated by other neighbouring loci.

These data offer some insight into the mechanisms of atherosclerosis, with the genotype predicted to lead to higher B₁R and B₂R expression/activity seemingly associated with reduced cardiovascular risk in the hypertensive state. KKS protection may be mediated through a number of potential mechanisms including B₁R-mediated vessel remodelling and angiogenesis, the inhibition of vascular smooth muscle cell growth, B₁R and B₂R-mediated coronary vasodilatation, and local nitric oxide synthesis. KKS activation also exerts a potent anti-thrombotic role. Factor XII activation and prekallikrein activation participate in a positive feedback loop, leading to rapid bradykinin release. This stimulates formation of tissue plasminogen activator and nitric oxide release, whilst kininogen breakdown products act as antithrombins. KKS activity therefore exerts antiadhesive, anticoagulant, and profibrinolytic effects, and can inhibit platelet activation at low thrombin concentrations. Thus, kinins (and, via this mechanism, the use of ACE inhibitors) may prevent coronary thrombosis. Genotype strongly influenced the cardiovascular risk associated with hypertension, whilst risk was genotype-independent amongst normotensives. Such a phenomenon may relate to a genotype-dependent difference in (potentially protective) receptor upregulation in the hypertensive state.
markers, through which 'classical' risk factors may partly mediate their effects. Hypertension is similarly associated with a systemic and local vascular inflammatory response which, through interleukin-driven activation of NF-κB, induces (potentially protective) vascular B₂R expression. Indeed, B₁ (and, to a lesser extent B₂) receptors are highly expressed in the atheromatous plaque. Whether B₂R expression may be similarly modulated is, however, not known.

These data extend our understanding of the mechanisms through which left ventricular hypertrophy and coronary artery disease are associated. Left ventricular hypertrophy (LVH) is an independent cardiovascular risk factor. Extensive animal data support a role for B₂R activation in diminishing this hypertrophic response, whilst the B₂R(+9) allele (associated with reduced receptor mRNA expression) is associated with an enhanced left ventricular hypertrophic response. The finding of a similar association of the B₂R(+9) allele with coronary artery disease amongst hypertensives suggests a novel common mechanism through which the deleterious effects of hypertension on left ventricular hypertrophy and CAD may be mediated. Further studies are required to elucidate any association of B₂R with the LV hypertrophic response.

These results also offer important insight into the mechanisms underlying the cardiovascular protective action of drugs which target the renin-angiotensin-system. Treatment with ACE inhibitors not only impairs kinin degradation, raising kinin levels in vivo, but also mediates cross-talk between membrane-bound ACE and the B₂R, leading to a reduction in B₂ desensitisation, reduced receptor endocytosis and an increase in bradykinin receptor affinity. Moreover, selective AT₁R blockade also raises tissue kinin levels and may influence crosstalk (through heterodimerization) between AT₁ receptors and bradykinin receptors. Our data would suggest that these phenomena may contribute to the marked vasculoprotective effects associated with ACE inhibition and, perhaps, with ARB use. Such data suggest potential gains in cardiovascular risk reduction from the combined use of ARBs with ACE inhibitors, or from the use of newer drugs, such as the neutral endopeptidase-ACE inhibitor class, which greatly increase kinin levels.

Although there was a higher frequency of current smoking amongst B₂R CC homozygotes (current smoking GG 28.7%, GC 34.0% and CC 44.4%; \( P=0.04 \)), this may be a chance observation. Certainly, allele frequencies do not suggest that a prior excess mortality amongst GG smokers could account for this observation (vide supra). However, the association of B₂R and B₂R polymorphisms with risk persists despite adjustment for smoking status, and indeed multifactorial adjustment for all other variables. Our observations are consistent with the previously-reported excess frequency of another B₂R polymorphism (B₂R–58C rather than –58T) amongst hypertensive (rather than normotensive) sufferers of acute MI. Thus, the consistent scientific rationale, and the strength of the observations made here suggest that the association of kinin receptor polymorphisms with coronary risk is robust, and strongly implicate the B₂R and B₂R genes (and their low expression) in determining high CAD risk amongst hypertensives. Although the interaction terms do not reach conventional levels of statistical significance, we believe that the biological significance of differences in risk is of interest and warrants confirmation in larger-scale prospective studies. In addition, the low frequency of the B₂R–699C allele did not allow for meaningful analysis of interaction between the two polymorphisms. Larger scale studies are also required to extend our observations from middle-aged Caucasian males to those of other racial origins and age ranges, as well as to women. Although case-control studies may be confounded by population stratification, the prospective study design of NPHSII is much more robust, and it is extremely unlikely that the association with risk we see here could be explained by such an effect. Population stratification could be ruled out by genotyping the samples for a group of randomly distributed single nucleotide polymorphisms (SNPs) but, to date, of the more than 40 SNPs where we have genotype data in NPHSII no statistically significant evidence of population stratification has been obtained.

A drawback of the study is that no detailed information about the specific cardiovascular medication received at baseline and after enrolment is currently available. However, the use of specific RAS antagonists was uncommon in the timeframe of study (NPHSII was started in 1989), since their putative role in primary prevention had not yet been elucidated. No treated hypertensives, nor patients with heart failure, were included at the onset of the study. The study was initiated well over 14 years ago and, at this time, these were the only two indications for therapy with ACE inhibitors, and ARBs were not yet available. Thus, there is no potential for pharmacogenomic interaction at outset. Similarly, the onset of heart failure (leading to ACEI treatment) would have necessitated development of one of the documented clinical endpoints (such as myocardial infarction), and as such could not have operated as a confounder. Meanwhile, the use for other reasons (such as diabetic nephropathy, or primary cardiovascular prevention) was not accepted during most of this timeframe and would, in any event, have applied to only a few individuals. Thus, it is unlikely that therapy with these (or other) agents could account for any differences observed, given that prescription would have had to have been strongly predicated by genotype to act as a significant confounder — and most indications for treatment would have been documented as a study endpoint. Evidently, however, one might postulate that hypertension itself may have been an active confounder leading to pharmacogenomic interaction. However, we see this as unlikely: analysis shows no association of the candidate genotypes with hypertension — whether defined as a categorical variable, or as a continuous trait. In support of the lack of confounding associations, the survival plots indicate divergence by genotype early on in the trial. Nonetheless, pharmacogenomic studies are warranted given the mechanistic implications of our data.
Finally, no candidate-gene investigation, including this one, can provide mechanistic data, and such commentary as provided above remains speculative. Further in vitro experimentation, together with studies in human disease and animal models, will be required to elucidate the roles of the B₁R and B₂R in the atherosclerotic process.

Acknowledgements

We would like to thank the British Heart Foundation for the support of SSD, JRP, SEH and HEM: FS/2001044, PG/02/021, RG2000 015, SP98003. The NPHS II study was supported by the Medical Research Council, the US National Institutes of Health (grant NHLBI 33014) and Du Pont Pharma. We should also like to thank the medical staff and patients who contributed to the NPHS II study and the Office for National Statistics (NHS) Central Registry for provision of mortality data.

References