Genes controlling postural changes in blood pressure: comprehensive association analysis of ATP-sensitive potassium channel genes KCNJ8 and ABCC9

Justine A. Ellis,1,2 Angela Lamantia,1 Raul Chavez,2 Katrina J. Scurrah,1,3 Colin G. Nichols,4 and Stephen B. Harrap1

1Department of Physiology, University of Melbourne, Melbourne; 2Murdoch Childrens Research Institute, Royal Children’s Hospital, Parkville; and 3Centre for Molecular, Environmental, Genetic and Analytic (MEGA) Epidemiology, University of Melbourne, Melbourne, Victoria, Australia; and 4Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri

Submitted 16 October 2009; accepted in final form 29 November 2009

Ellis JA, Lamantia A, Chavez R, Scurrah KJ, Nichols CG, Harrap SB. Genes controlling postural changes in blood pressure: comprehensive association analysis of ATP-sensitive potassium channel genes KCNJ8 and ABCC9. Physiol Genomics 40: 184–188, 2010. First published December 1, 2009; doi:10.1152/physiolgenomics.00173.2009.—Buffering of blood pressure during change of posture such as standing is controlled largely by the baroreflex. In our population-based Victorian Family Heart Study (VFHS), we previously demonstrated that, on average, systolic blood pressure (SBP) changes very little on standing; however, interindividual variation is substantial and shows familial aggregation, with ~25% of the variance attributable to genetic factors. Our genomewide linkage analysis suggests a region on chromosome 12p that harbors two strong candidate genes, KCNJ8 and ABCC9, encoding the channel-forming inward rectifier subunit Kir6.1 and the ATP-sensitive binding cassette SUR2B, respectively. These are key components of smooth muscle ATP-sensitive potassium (KATP) channels, important regulators of arterial tone and blood flow and central to autonomic baroreceptor control of changes in total peripheral resistance. We performed a comprehensive association analysis of 47 tag single nucleotide polymorphisms (SNPs) spanning the KCNJ8 and ABCC9 gene regions with postural change in SBP (∆SBP). To augment power, we took a selective genotyping approach in which we compared allele and genotype frequencies between 150 unrelated individuals with high (positive) ∆SBP (≥7 mmHg) and 150 unrelated individuals with low (negative) ∆SBP (≤−7 mmHg) drawn from the offspring generation (18–30 yr) of the VFHS. Association analyses showed that no SNPs demonstrated statistically significant differences in genotype frequencies between groups, particularly after adjustments for multiple testing. We conclude that sequence variants in KCNJ8 and ABCC9 are unlikely to contribute to variation in ∆SBP. Other genes in the identified chromosome 12p region warrant investigation.

baroreflex; baroreceptors; single nucleotide polymorphisms; Kir6.1; SUR2B

HOMEOSTASIS OF BLOOD PRESSURE is of fundamental physiological importance to ensure relative stability of organ blood flow under a variety of normal daily activities and periods of hemodynamic stress. The baroreflex is a key short-term blood pressure control mechanism that depends on sensors (baroreceptors) located in key arteries such as the carotid, central neural control in the brain stem, and the innervation of the heart and blood vessels by the autonomic nervous system (27). The baroreflex is responsible for buffering disturbances in blood pressure via alterations to cardiac output and peripheral arterial resistance (25). Abnormalities of the baroreflex have been associated with hypertension (20), orthostatic hypotension (15), chronic renal failure (25), and long-term cardiovascular risk.

The change in blood pressure that occurs with a change to posture such as standing is a simple test of the baroreflex. Population-based studies such as our Victorian Family Heart Study (VFHS) (10) have demonstrated that, on average, systolic blood pressure (SBP) changes very little on standing (9). However, at an individual level, the magnitude and direction of the change are significantly variable and show familial aggregation, with ~25% of the total variance determined by genes (9). Given the important role of the baroreflex in buffering postural blood pressure changes, genes relevant to baroreflex sensitivity are strong candidates for control of blood pressure with change of posture.

We previously performed (9) a genomewide linkage scan to identify regions of the genome with evidence of involvement in control of postural change in SBP (∆SBP). A region on chromosome 12p demonstrated suggestive linkage, prompting investigation of the candidate genes under the linkage peak. The region harbors >400 genes; however, two adjacent genes, KCNJ8 and ABCC9, stand out as excellent candidates. These genes encode the pore-forming inward rectifier subunit Kir6.1 and the ATP-sensitive binding cassette SUR2B, respectively. Together, these are key components of smooth muscle ATP-sensitive potassium (KATP) channels (29). KATP channels are prominent in vascular smooth muscle cells and produce smooth muscle relaxation (vasodilation) when activated (12). They are thus important regulators of arterial tone and blood flow and central to the effector arm of the autonomic baroreceptor control of changes in total peripheral resistance. Rodent knockout models of these two genes demonstrate phenotypes characterized by systemic hypertension and coronary vasospasm, mimicking human Prinzmetal (variant) angina (3, 18), raising the possibility that more subtle abnormalities of gene expression might be manifest as abnormalities of vascular tone that become evident in situations in which precise response to baroreflex commands is required. For example, KATP channel activity has been shown to modulate sympathetic vasoconstriction during exercise (13).
The K\textsubscript{ATP} channel may also be relevant to the afferent arm
of the baroreflex. In mammals, the genes \textit{KCNJ8} and \textit{ABCC9}
form part of a very small locus on chromosome 12p that has
been linked with a Mendelian form of hypertension in which
diminished baroreflex sensitivity is a feature (16). Studies
using hydrogen sulfide (H\textsubscript{2}S), an activator of K\textsubscript{ATP} channels
(33), indicate that this channel may play a role in normal
function of the baroreceptors: rats given H\textsubscript{2}S showed facilita-
tion of carotid baroreceptor activity such that carotid sinus
discharge was higher for a given blood pressure and
was more sensitive to changes in blood pressure (32).

To determine the role of sequence variation in and around
\textit{KCNJ8} and \textit{ABCC9} in regulating \textit{SBP}, we performed a
comprehensive tag single nucleotide polymorphism (SNP)-
based genetic association analysis. We used a selective geno-
typing approach whereby we compared tag SNP genotypes of
150 unrelated individuals from the offspring generation (aged
18–30 yr) of the VFHS in whom SBP rose most on standing to
150 such individuals in whom SBP fell most on standing, to
identify variants relevant to the phenotype. This approach has
been demonstrated to be a powerful and useful method when
applied to studies of at least 1,000 individuals sampled at
random from a population (31).

MATERIALS AND METHODS

\textbf{Subject recruitment and phenotyping.} Subjects were drawn from
the VFHS, a population-based study of 2,911 healthy adults recruited
between 1991 and 1996 (10). The VFHS comprises 767 families
consisting of two parents (aged 40–70 yr) and at least one natural
offspring (aged 18–30 yr). Recruitment was limited to Caucasian
families. A history of heart disease was not relevant to recruitment,
the aim being to enroll a representative sample of subjects exhibiting
a broad range of cardiovascular risk factors. These studies were
approved by the Ethics Review Committee of the Alfred Hospital,
Melbourne, Australia, and informed consent was obtained from all
participants (10).

After the subjects rested for 10 min, three measures of SBP were
taken in the supine position, the last two of which were recorded.
Subjects then stood for 2 min, and a further three measurements of
SBP were made, the last two of which were recorded. \textit{ASBP} was
calculated as the average of the recorded standing pressures minus
the average of the recorded supine pressures (9, 26).

A blood sample was taken from all participants, and DNA was
extracted and stored (10).

\textbf{Subject selection.} For this study we selected from the
offspring generation only \((n = 1,377). The reasons for this were
threefold. First, the linkage signal on chromosome 12 was detected
through analysis of offspring only (9). Second, the use of antihyper-
tensive and lipid-lowering drugs was significantly less common in the
offspring generation, avoiding the confounding effects of these drugs
on \textit{SBP} measures. Third, the use of the younger generation avoids any
confounding effects of aging on the baroreflex. Within the offspring
generation, mean \textit{ASBP} was 0.45 mmHg (\textit{SD} = 7.3).

To maximize potential genetic differences and statistical power to
identify variants relevant to the determination of \textit{ASBP}, we used a
selective genotyping approach to subject selection (2, 22, 31). High
(positive) \textit{ASBP} was defined as \textit{ASBP} greater than or equal to 7
mmHg (i.e., \(-1 SD\) away from the mean). Low (negative) \textit{ASBP} was
defined as \textit{ASBP} less than or equal to \(-7\) mmHg. We selected 150
unrelated offspring individuals with high (positive) \textit{ASBP} and 150
unrelated offspring individuals with low (negative) \textit{ASBP}. The contrast-
mutations groups contained equal numbers of male and female
individuals.

\textbf{Tag SNP selection.} Tag SNPs were selected to comprehensively
capture SNP variation in the region spanning \textit{KCNJ8} and \textit{ABCC9}
and all intergenic regions from these genes to the adjacent genes in the
5’ and 3’ flanking regions (Fig. 1). The region examined was \(>388\) kb
in length, from Chr12:21702042 to Chr12:22090426. The Interna-
tional HapMap database (28) (Phase II, //www.hapmap.org) was used
to identify SNPs in this region, and then HapMap linkage disequi-
librium (LD) data were downloaded and interrogated in the Haplovie-
version 4.0 (1) program. Tag SNPs were selected with Tagger (4)
within Haplovie, and SNPs with a minor allele frequency (MAF)
\(\geq 1\%\) were included in the selection. A total of 47 tag SNPs were
chosen that represent 244 SNPs in the region with a minimum LD \(r^2\)
value of 0.7. These SNPs and their chromosomal location are shown in
Fig. 1.

\textbf{Genotyping.} Genotyping was performed in a blinded fashion across
three platforms as they became available to the project. A total of six
tag SNPs were genotyped on the MegaBACE 1000 DNA Analysis
Platform (GE Biosciences) with single nucleotide primer extension
chemistry (SNuPE, GE Biosciences) according to manufacturer pro-
tocols. Primers used for the PCR and extension reactions are shown in
Supplemental Table S1.\footnote{The online version of this article contains supplemental material.} Genotypes were called with MegaBACE
SNP Profiler v1.0 software (GE Biosciences). A total of two tag SNPs
were individually genotyped on the LightScanner High Resolution
Melt (HRM) system (Idaho Technologies). Unlabeled probe genotyp-
ing using LCGreen Plus fluorescent dye (Bio-Rad) was performed
with primers designed for each specific SNP and LightScanner Primer
Design Software (Idaho Technologies). PCR primers and probe se-
quences are shown in Supplemental Table S2. The remaining 39 tag
SNPs were genotyped with the Sequenom MassARRAY matrix-
assisted laser desorption/ionization-time of flight (MALDI-TOF)
genotyping system using Sequenom iPLEX Gold chemistries accord-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Locations of the tag single nucleotide polymorphisms genotyped to comprehensively span the \textit{KCNJ8} and \textit{ABCC9} genes and flanking intergenic regions. Figure prepared with Haploview (1).}
\end{figure}
RESULTS

The phenotypic characteristics of the overall VFHS offspring group, and those of the 150 high ΔSBP and 150 low ΔSBP offspring subjects selected for this study, are presented in Table 1. No appreciable differences in age, height, weight, BMI, SBP, or diastolic blood pressure were evident among the groups, demonstrating that the selected groups were phenotypically representative of the entire VFHS offspring group with the exception of ΔSBP. All 300 selected subjects were genotyped for the 47 tag SNPs spanning the genomic region of interest. Eighteen subjects (8 high and 10 low ΔSBP) were removed from further analyses because the genotype call rate fell below 90%. After removal of these subjects, all remaining subjects were genotyped with a successful call rate of >90% (overall genotyping rate of 99%).

Five SNPs (rs704175, rs829080, rs12230539, rs704190, rs3809205) were found to deviate from Hardy-Weinberg equilibrium (HWE) was observed in one group but not the other. Such distortions in genotype frequency can be an indication of association (5, 14). Two SNPs (rs1352908, rs704199) were found not to be polymorphic in our population. Thus alleles and genotypes from a total of 45 SNPs were compared between the high ΔSBP and low ΔSBP phenotypic groups.

All SNPs were initially analyzed with allelic and genotypic χ²-tests. For SNPs where the minor allele homozygote counts were <5 (rs2418004, rs11046163, rs11046179, rs17697582, rs11046209, rs11836934, rs289080, rs12230539, rs11046217, rs11836595, rs4148663, rs1283802, rs1352909, rs2900492, rs11046232, rs4148649, rs4762865, rs10505874, rs17420883, rs9645737, rs10770876, rs11046263, rs11046268, rs3809205, rs10770881), we applied Fisher’s exact test to obtain a genotypic 𝑃 value. The results of the χ²-analyses are shown in Supplemental Table S4.

Adjustments for multiple testing were done in two ways. First, although considered overly stringent for studies such as these where SNPs are not entirely independent, a simple Bonferroni correction for 45 tests (0.05/45) sets the significance 𝑃 value for all SNPs at 𝑃 = 0.001. No 𝑃 value obtained in this study reached or near this level of significance. The empirical 𝑃 values obtained from 1,000 permutations (p_perm) were calculated for each χ²-test, and these are shown in Supplemental Table S4. After adjustment for multiple testing, no tag SNPs in the ABCC9–KCNJ8 genomic region were found to be associated with ΔSBP.

Nevertheless, additive and genotypic logistic regression analyses were performed for SNPs showing some evidence of association before multiple testing adjustments. Allelic χ² comparisons for SNP rs2955503 were significant at the 𝑃 < 0.05 level (𝑃 = 0.043). This SNP remained associated by additive (but not genotypic) logistic regression analysis that adjusted for the covariates age, sex, and BMI but did not remain associated after adjustment for multiple testing [additive: odds ratio (OR) = 1.43, 95% confidence interval (CI) 0.99–2.04, 𝑃 = 0.055, p_perm = 0.79; genotypic (2df): 𝑃 = 0.10, p_perm = 0.43, OR for heterozygotes compared with major allele homozygotes = 1.17, 95% CI 0.70–1.96 (𝑃 = 0.55), OR for minor allele homozygotes compared with major allele homozygotes = 2.38, 95% CI 1.07–5.27 (𝑃 = 0.033)]. Allelic χ² comparisons for SNP rs2900492 were also significant (𝑃 = 0.020). The SNP remained associated by logistic regression adjusting for the covariates above (no minor allele homozygotes observed; OR for heterozygotes compared with major allele homozygotes = 4.29, 95% CI 1.18–15.69, 𝑃 = 0.028) but did not remain associated after multiple testing adjustment (p_perm = 0.56).

It is possible that there exist rare alleles that might exert modest effects on postural changes in blood pressure. These might be captured by the rare haplotypes that result between tag SNPs, which by their very nature show little LD. As a subsidiary exploratory analysis we performed haplotype analyses both in a pairwise fashion with rs2955503 and rs2900492 and with a sliding window approach that included 2, 3, or 4 SNPs in each window. Haplotypes were estimated with the standard E-M algorithm in PLINK, and differences between cases and controls were assessed by χ²-analyses. The pairwise haplotype association was not materially different from the association seen for each SNP alone (best: rs2900492 A, rs2955503 A; estimated frequency in cases 56%, controls 66%;

Table 1. Phenotypic characteristics of entire VFHS offspring group, high (positive) ΔSBP VFHS offspring group, and low (negative) ΔSBP VFHS offspring group

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>All VFHS (n = 1,377)</th>
<th>High (positive) ΔSBP VFHS Offspring Group (n = 150)</th>
<th>Low (negative) ΔSBP VFHS Offspring Group (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSBP, mmHg</td>
<td>0.45 ± 7.3</td>
<td>11.6 ± 4.7</td>
<td>−12.6 ± 4.1</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24.0 ± 3.7</td>
<td>24.3 ± 3.7</td>
<td>23.9 ± 3.8</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>47.7</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.8 ± 13.7</td>
<td>70.9 ± 14.5</td>
<td>67.7 ± 13.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171.0 ± 9.3</td>
<td>171.8 ± 9.2</td>
<td>171.0 ± 9.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4 ± 3.7</td>
<td>23.9 ± 3.7</td>
<td>23.0 ± 3.5</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>117.5 ± 11.2</td>
<td>119.4 ± 9.9</td>
<td>116.8 ± 11.2</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>70.0 ± 9.2</td>
<td>71.8 ± 8.9</td>
<td>66.8 ± 10.1</td>
</tr>
</tbody>
</table>

All phenotype data (except sex) are shown as means ± SD. VFHS, Victorian Family Heart Study; SBP, systolic blood pressure; ΔSBP, change in SBP; DBP, diastolic blood pressure; BMI, body mass index.
POSTURAL BLOOD PRESSURE CHANGES, KCNJ8, AND ABCC9

P = 0.025). Sliding window haplotypes containing either rs2955503 or rs2900492 generally showed weak association, but again these were not materially different from the associations seen for each SNP alone (best: rs2900492 G, rs11046232 A, rs4148649 A, rs4762865 A; estimated frequency in cases 4%, controls 1%; P = 0.019). We treat these results with caution in view of the limited power of this study (even with selective sampling from the extremes of the distribution) and the fact that in the absence of information regarding phase, such rare haplotypes are inferred only. Larger samples and direct sequencing might be a more appropriate approach to rare alleles, but our findings provide little evidence to pursue such an approach at this stage.

DISCUSSION

To the best of our knowledge, this is the first study to perform genewide association analyses of candidate genes for ΔSBP. Changes in blood pressure induced by alterations to posture are indicative of the sensitivity of the baroreflex. If the changes to blood pressure are minimal, as is the case, on average, in the general population, then the baroreflex sensitivity and response is optimal. However, if changes in blood pressure from lying to standing are large, as can be seen at an individual level (9), this is likely to indicate a less than optimal baroreflex response. The buffering ability of the baroreflex has been associated with a number of clinical conditions including hypertension (20) and orthostatic hypotension (15). Uncorrected reductions in SBP on standing might predispose to reduced cerebral perfusion and symptomatic postural hypotension (8) or ischemic damage. Large increases in pressure on standing might augment hemodynamic stress and predispose to vascular damage. The Atherosclerosis Risk in Communities (ARIC) Study suggested that subjects at the extremes of the distribution for the postural change in SBP are more likely to be hypertensive (23) and experience coronary heart disease and stroke (6, 24).

We have shown in the past that ΔSBP has a heritable component, and we generated evidence, through genomewide linkage analyses in the offspring generation of the VFHS, that a locus regulating ΔSBP may lie on chromosome 12 (9). Investigation of the region under the linkage peak identified two strong candidate genes for involvement in regulation of the baroreflex, KCNJ8 and ABCC9, encoding key components of vascular K_{ATP} channels (29). We performed a comprehensive association study of tag SNPs representing variation in, and around, these two genes by comparing allelic and genotypic frequencies of each tag SNP between individuals from the VFHS offspring generation who demonstrated high (positive) ΔSBP and individuals who demonstrated low (negative) ΔSBP. We found no evidence, particularly after adjustment for multiple testing, of association of variants captured by our tag SNPs and haplotypes in these gene regions with ΔSBP, consistent with earlier, more narrow studies that failed to detect association of KCNJ8 or ABCC9 variants either with abnormal coronary vasomotion (7) or with coronary angina (30).

Our analyses involved comparison of 150 individuals with high (positive) ΔSBP with 150 individuals with low (negative) ΔSBP. By today’s association study standards, these numbers may at first appear small. However, we took a selective genotyping approach to subject selection, in order to maximize phenotypic, and therefore genotypic, contrast and thus maximize power to detect differences at the genotypic level (2, 22, 31). We estimate that our total sample size of 1,377 related individuals from the offspring generation is approximately equivalent to 1,100 unrelated individuals, from which we then selected the highest 14% and the lowest 14%. Using previously published estimates (31) we estimated that we had excellent power (>99%) to detect effects that explain at least 5% of the total variance under a dominant or an additive model for most allele frequencies (0.1–0.9) and satisfactory power (at least 70%) to detect effects that explain at least 1% of the variance under a dominant model (for allele frequencies of 0.1–0.9) or under an additive model (for allele frequencies of 0.1–0.2).

While our approach to the selection of HapMap tag SNPs across the KCNJ8 and ABCC9 genomic region provides very good coverage of common sequence variation, the potential presence of causal variants not in LD with any of the tag SNPs or haplotypes examined cannot be entirely excluded. Such variants might be rare and therefore may only be identified by extensive resequencing procedures, which were beyond the scope of this study. In the absence of evidence of association of KCNJ8 and ABCC9 with ΔSBP by our approach, other candidate genes in the chromosome 12 linkage region might now be considered. Among other genes, further candidates include the ion channel gene CACNB3 (voltage-dependent calcium channel β3-subunit) (19) and the brain sodium channel ACCN2, a member of the degenerin (DEG)/epithelial sodium channel (ENaC) superfamily (17). Also found in this region is the gene encoding the adrenomedullin (ADM) receptor, ADMR. ADM is a vasodilatory peptide that has recently been shown to enhance baroreflex response via ADM receptors in the brain of rodents (11). It remains to be seen whether genetic variation in these genes regulates ΔSBP via the baroreflex in humans.

In summary, we have performed a follow-up study to previous work confirming a heritable component to postural ΔSBP and evidence of genetic variation on chromosome 12 that regulates ΔSBP. The chromosome 12 candidate genes KCNJ8 and ABCC9 have been demonstrated by this study to be unlikely to harbor genetic variation that regulates the change in SBP from lying to standing. Other genes in the region now warrant further investigation. Uncovering the genes relevant to the regulation of postural changes to blood pressure will provide insight into the regulation of the baroreflex and blood pressure in general, and into the genetic physiology of clinical conditions relevant to baroreflex buffering, such as orthostatic hypotension.

ACKNOWLEDGMENTS

We thank Margaret Stebbing, Prof. John Hopper, Prof. Graham Giles, and the general practitioners and research nurses for their contributions to recruitment of VFHS participants. We acknowledge the assistance of the Murdoch Childrens Research Institute Sequenom Platform Facility within which the majority of the genotyping for this study was carried out.

GRANTS

This work was supported by a Grant-in-Aid from the National Heart Foundation of Australia. J. A. Ellis is supported by a National Health and Medical Research Council (Australia) Capacity Building Grant in Population Health. K. J. Scurrah acknowledges support from the National Health and Medical Research Council (Australia).

DISCLOSURES

The authors declare no conflict of interest.
REFERENCES


