

# The BK channel $\beta 1$ subunit gene is associated with human baroreflex and blood pressure regulation

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**Background** The baroreflex, which is important for the minute-to-minute regulation of blood pressure and heart rate, is influenced by genetic variance. Ion channels are important to baroreflex afferent and efferent function. Mice missing the  $\beta 1$  subunit of the  $\text{Ca}^{2+}$ -sensitive potassium channel (BK) are hypertensive and have a reset baroreflex. We tested the hypothesis that variants in the gene (KCNMB1) coding for the BK  $\beta 1$  subunit are associated with baroreflex function.

**Methods** We studied six single-nucleotide polymorphisms (SNPs) in KCNMB1.

**Results** Four SNPs in intron 3, exon 4a, exon 4b and exon 4c gave significant results. For instance, exon 4b SNP AA individuals had higher heart rate variability, compared to CA, or CC persons, in particular in the high-frequency range. The low-frequency range showed no association. Consistent with the heart rate variability data, homozygous AA persons had greater baroreflex slopes than CA or CC persons, also in the high-frequency range. These associations could not be shown in the low-frequency range for heart rate variability and baroreflex slopes.

## Introduction

Large-conductance, voltage and  $\text{Ca}^{2+}$ -sensitive potassium channels (BK) are important to calcium-mediated relaxation. The channels are expressed in vascular smooth muscle cells and are important in regulating arteriolar tone. However, the channels are also expressed in neuronal tissue and thereby may influence vascular regulation. The BK channel subunits ( $\alpha$  and  $\beta 1$ ) are activated by calcium sparks [1]. Spatially,  $\text{Ca}^{2+}$  transients can be global, involving the entire cell, or they can be restricted to a limited area of the cytosol, namely calcium sparks.  $\text{Ca}^{2+}$  sparks have a local regulatory function. They are caused by the opening of small ryanodine-sensitive  $\text{Ca}^{2+}$  release channel clusters localized in the sarcoplasmic reticulum. Brenner *et al.* [2] and Plüger and colleagues [3] showed independently that mice with disrupted  $\beta 1$  subunit genes exhibit a decrease in BK channel calcium sensitivity and have an increased blood pressure, compared to

\*The contributions of M.G. and J.T. were equal; those of A.B. and S.B. were also equivalent.

**Conclusions** These data support the notion that variants in channel genes may be responsible for the great range in heart rate variability and baroreflex function observed in humans. Such variation may also play a role in the development of hypertension. *J Hypertens* 20:927–933 © 2002 Lippincott Williams & Wilkins.

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wild-type mice. These observations make the  $\beta 1$  subunit an attractive candidate gene for blood pressure regulation in humans. We recently observed that not only resting blood pressure, but also baroreflex blood pressure regulation, are strongly influenced by genetic variance [4]. Here we have employed the twin model and tested the hypothesis that the human  $\beta 1$  BK channel subunit gene (KCNMB1) plays a role in blood pressure regulation in normal humans.

## Methods

We recruited 88 pairs of normal monozygotic (MZ) and 61 pairs of normal dizygotic (DZ) twins to participate in studies involving blood pressure regulation and cardiovascular phenotypes, as described earlier [5,6]. The zygosity was verified with the use of five polymerase chain reaction (PCR)-amplified microsatellite markers, as described in detail elsewhere [7]. The subjects were all German Caucasians recruited from various parts of Germany. The university's committee on the protection of human subjects approved the protocol and

written informed consent was obtained from all participants.

The protocols for determining baroreflex function have been described previously [4,8,9]. The studies were conducted in a quiet room at 20°C during morning hours, with subjects in a semi-supine body position. Briefly, 5-min recordings were obtained after at least 10 min of rest. Blood pressure was measured in the non-dominant arm by an automated oscillometric device (Dinamap), as well as continuously at the middle finger of the right hand by the Finapres (Ohmeda) blood pressure monitor. The hand was kept at heart level. An electrocardiogram (ECG) was recorded continuously using a modified standard lead to optimize the R-peak. Heart rate variability was determined in the time and frequency domain using standard techniques. The square root of the mean squared differences of successive normal-to-normal intervals (RMSSD) and the proportion of successive normal-to-normal intervals differences greater than 50 ms (pNN50) were calculated. We calculated the power spectra of systolic blood pressure and the electrocardiographic R-wave to R-wave (RR) interval time series. The baroreflex RR slope was determined by cross-spectral analysis and by the sequence technique.

To capture the common underlying trait and thereby reduce the multiple testing problem, we calculated a factor score based on the correlation between the different measures of heart rate variability, blood pressure variability and baroreflex sensitivity. We relied on a principal components analysis. The major factor derived from this analysis captures 57% of the total variance and is related to all heart rate variability and baroreflex sensitivity measures. A second factor captured 17% of the total variance and was composed of low-frequency systolic blood pressure variability (LFSBP) and high-frequency systolic blood pressure variability (HFSBP). For the genetic analysis we

decided to rely on the first major factor for testing significance, while in a *post-hoc* analysis we included all 10 physiological measures for an exploratory analysis to guide hypothesis building as to the mode of action of the genetic influence.

#### Single-nucleotide polymorphism (SNP) detection

The genomic organization of the BK channel  $\beta 1$  subunit has been described earlier [10]. Genomic DNA was extracted from peripheral blood by using standard methods. Two of the six SNPs (exon 1: dbSNP cluster ID rs03508; intron 2: dbSNP cluster ID rs03507) were identified by Database search ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?locusId=3779](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3779)). Sequencing of the four KCNMB1 exons with flanking intronic primers in 30 unrelated individuals resulted in identification of SNPs in exon 4 (termed exon 4a, 4b, 4c). PCR and minisequencing primer design was based on the sequence of clone RP11-292M11 (GeneBank accession no. AC027741). Exon 4 was subdivided in three amplicons 4.1, 4.2, 4.3. Primer sequences are listed in Table 1. Detailed methods for our SNP analyses have been published [11].

#### Association

We evaluated six SNPs in the KCNMB1 gene, one in exon 1, one in intron 2, one in intron 3 and three in exon 4. Descriptive statistics for the SNPs showed an additive mode of action. Phenotypic values were compared between groups defined by their genotype using ANOVA for all polymorphisms independently. As this approach might be prone to false-positive results due to population stratification, a second analysis based on DZ sib-pair data was carried out [12]. A true allelic effect will affect the difference between phenotypic measures within a sib-pair, as well as the difference between mean values of sib-pairs, depending on their genotypes. Members of a sib-pair arise from the same stratum of the population. Thus, estimates of the allelic effect based on within-family differences are not prone to

Table 1 The primer sequences

PCR primers					
SNP	5'-Primer	3'-Primer	Minisequencing primer	Strand	Variation
Exon 1	TTT CCA AAT ATA CCA GGC TGA TCT TTC	CAG GCT GCT TTC CCC TGT CTC	CAG GCA GAA AGA AAC	+	C/T
Intron 2	ACA ATT CCT CCC TGT GTC CGG G	CAG GGA CAG TTA GGA ACA GGC TCA C	GCT GAA GAC AAG ATG AGG A	+	T/G
Intron 3	ACT ACA AAG CTG GTA TTT TTC TTT ACT CCA	GCC AGA AGA GGG AGA AGA GGA	GGG ATT TCC AAG CCC AC	+	G/A
Exon 4a	ACT ACA AAG CTG GTA TTT TTC TTT ACT CCA	GCC AGA AGA GGG AGA AGA GGA	CCT ACA TCC CAG GCA GC	+	G/C; V110L
Exon 4b	AAC GAA ACC AGC GTC CTA TTC C	AAT AGA GAG CTA GAA CTG GCT GGC	GCT GCT CCC CAC TTG CAG	+	C/A
Exon 4c	AAC GAA ACC AGC GTC CTA TTC C	AAT AGA GAG CTA GAA CTG GCT GGC	GCA GGT GGA GAA GGC ATT G	-	G/T

PCR, polymerase chain reaction; SNPs, single-nucleotide polymorphisms.

stratification influences. To test for association, structural equation modelling was used to obtain maximum likelihood estimates for the allelic effect, based on within-family and between-family differences. Significance was tested by computing nested models and comparing the log-likelihood between models. To test for stratification effects, both estimates of allelic effect are then constrained to be equal. In the absence of stratification, both estimates were set to zero. In the presence of stratification effects, setting only the within-family estimate to zero would provide an unbiased test for true allelic association. Analysis was done by using a structural equation modelling approach as implemented in the MX-package [13]. Since we used a candidate gene approach, we accepted  $P < 0.01$  to test for significant linkage, in accordance with the criteria defined by Lander and Kruglyak [14]. As the genetic markers within a gene are likely to be in tight disequilibrium, we employed a backward stepwise regression analysis to define the minimal set of SNPs needed to predict the phenotype.

## Results

Table 2 provides descriptive statistics and heritability estimates as described earlier [4]. The DZ twins and the MZ twins were not different in terms of their baroreceptor responses. New variables are low-frequency RR variability (LFRR), high-frequency RR variability (HFRR), and the same for systolic (LFSBP and HFSBP) blood pressure. Baroreflex slopes in the low- and high-frequency band (BRSHF and BRSLF)

were determined with a cross-spectral analysis. Baroreflex slope, BRS+, are the up-sloping segments while BRS- are the down-sloping segments, as determined by sequence analysis.

The SNPs were interdependent and were in linkage disequilibrium with one another, with the exception of the SNP in exon 1. Table 3 gives the results of the association analysis. The factor given encompasses all the information available on heart rate variability and baroreflex sensitivity. The result indicates highly significant associations, particularly with the SNP in intron 3 and in exon 4b and 4c. Stepwise regression analysis showed that one SNP was sufficient to explain most of the genetic effect on heart rate variability and baroreflex sensitivity. The exon 4b SNP had the highest predictive value. We performed a *post-hoc* analysis of the separate components that were included in the factor analysis, as shown in Table 4. We found significant association with pNN50 and RMSSD, which are established measures for heart rate variability. Spectral analysis revealed that the effect was limited to high-frequency oscillations of RR variability. In contrast, we did not find a significant association for RR variability in the low-frequency band. The SNP also had an effect on the baroreflex RR slope determined using the sequence technique (up + and down - slopes). Moreover, we found a significant association for baroreflex RR slopes in the high-frequency band determined by cross-spectral analysis. There was no significant association for baroreflex RR slopes in the low-frequency

**Table 2** Descriptive statistics, intraclass correlation  $r$  and heritability  $h^2$

Parameter	MZ mean $\pm$ SD	DZ mean $\pm$ SD	$r_{MZ}$	$r_{DZ}$	$h^2$
Number	176	122			
Age (years)	33 $\pm$ 13	33 $\pm$ 11			
Gender (F/M), $n$	116/60	82/40			
Height (cm)	169 $\pm$ 9	170 $\pm$ 9			
Weight (kg)	67 $\pm$ 11	70 $\pm$ 15			
BMI (kg/m <sup>2</sup> )	23.0 $\pm$ 3.5	23.8 $\pm$ 4.0			
SBP (mmHg)	125 $\pm$ 16	123 $\pm$ 13			
DBP (mmHg)	72 $\pm$ 11	73 $\pm$ 10			
Heart rate (bpm)	71 $\pm$ 11	69 $\pm$ 12			
RMSSD	63 $\pm$ 47	59 $\pm$ 43	0.30	0.34	0
pNN50	22 $\pm$ 20	20 $\pm$ 19	0.33	0.24	0.18
LFRR	1271 $\pm$ 1446	1310 $\pm$ 1640	0.29	0.09	0.28
HFRR	940 $\pm$ 1883	734 $\pm$ 1179	0.15	0.03	0.14
LFSBP	7.0 $\pm$ 8.6	7.5 $\pm$ 7.5	0.18	0.00	0.16
HFSBP	2.3 $\pm$ 2.3	1.6 $\pm$ 1.3	0.29	0.21	0.13
BRSLF	16 $\pm$ 13	16 $\pm$ 9	0.48	0.05	0.43
BRSHF	21 $\pm$ 17	21 $\pm$ 14	0.44	-0.03	0.40
BRS +	19 $\pm$ 15	18 $\pm$ 10	0.46	0.03	0.42
BRS -	21 $\pm$ 15	20 $\pm$ 11	0.38	0.09	0.36
Factor 1	0.07 $\pm$ 1.13	0 $\pm$ 0.82	0.48	0.14	0.47

MZ, monozygotic; DZ, dizygotic; F/M, female/male; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RMSSD, square root of the mean squared differences of successive normal-to-normal intervals; pNN50, proportion of successive normal-to-normal intervals differences greater than 50 ms; RR, R-wave to R-wave; LFRR, low-frequency RR variability; HFRR, high-frequency RR variability; LFSBP, low-frequency systolic blood pressure variability; HFSBP, high-frequency systolic blood pressure variability; BRSLF, baroreflex slopes in the low-frequency band; BRSHF, baroreflex slopes in the high-frequency band; BRS+, baroreflex slope s, upsloping segments; BRS-, baroreflex slope s, down-sloping segments.

**Table 3 Association results based on the factor containing heart rate variability and baroreflex sensitivity. P values are given for the ANOVA on DZ + MZ data as well as for the DZ-based maximum likelihood sib-pair analysis (ML sibs)**

Location	Genotype	N	Mean ± SD	P ANOVA	P ML sibs
Exon 1	TT	0		0.284	0.980
	CT	25	-0.190 ± 0.493		
	CC	102	0.007 ± .0879		
Intron 2	GG	35	0.061 ± 1.045	0.359	0.092
	GT	64	-0.012 ± 0.681		
	TT	27	-0.233 ± 0.824		
Intron 3	GG	18	0.506 ± 1.198	0.003	0.010
	AG	61	-0.054 ± 0.666		
	AA	47	-0.243 ± 0.743		
Exon 4a	CC	2	-0.383 ± 0.230	0.027	0.373
	GC	26	0.307 ± 1.030		
	GG	100	-0.122 ± 0.744		
Exon 4b	AA	17	0.620 ± 1.170	0.004	0.007
	CA	62	-0.090 ± 0.674		
	CC	47	-0.201 ± 0.763		
Exon 4c	GG	18	0.560 ± 1.164	0.006	0.010
	GT	61	-0.084 ± 0.678		
	TT	48	-0.207 ± 0.756		

MZ, monozygotic; DZ, dizygotic

**Table 4 Association results for exon 4b and the various baroreflex slope measures**

Phenotype	Genotype	n	Mean	P ANOVA	P ML sibs
RMSSD	AA	17	83.2 ± 56.4	0.017	0.017
	CA	62	58.4 ± 38.5		
	CC	47	49.7 ± 37.4		
pNN50	AA	17	35.4 ± 22.2	0.001	0.004
	CA	62	19.0 ± 16.2		
	CC	47	16.0 ± 18.4		
LFRR	AA	17	1700 ± 1807	0.438	0.886
	CA	62	1133 ± 1374		
	CC	47	1286 ± 1801		
HFRR	AA	17	1678 ± 2601	0.001	0.016
	CA	62	575 ± 492		
	CC	47	542 ± 774		
LFSBP	AA	17	7.14 ± 6.60	0.988	0.718
	CA	62	7.43 ± 7.94		
	CC	47	7.44 ± 6.60		
HFSBP	AA	17	1.27 ± 0.55	0.372	0.133
	CA	62	1.83 ± 1.98		
	CC	47	1.96 ± 1.70		
BRSLF	AA	17	18.5 ± 8.2	0.233	0.737
	CA	62	14.4 ± 8.4		
	CC	47	15.5 ± 9.3		
BRSHF	AA	17	33.1 ± 21.8	0.000	0.002
	CA	62	20.0 ± 10.5		
	CC	47	17.2 ± 11.7		
BRSPS	AA	17	23.9 ± 14.1	0.010	0.013
	CA	62	16.8 ± 9.0		
	CC	47	15.5 ± 8.9		
BRSNS	AA	17	26.1 ± 14.3	0.005	0.002
	CA	62	19.4 ± 10.9		
	CC	47	16.1 ± 8.8		

RMSSD, square root of the mean squared differences of successive normal-to-normal intervals; pNN50, proportion of successive normal-to-normal intervals differences greater than 50 ms; LFRR, low-frequency RR variability; HFRR, high-frequency RR variability; LFSBP, low-frequency systolic blood pressure variability; HFSBP, high-frequency systolic blood pressure variability; BRSLF, baroreflex slopes in the low-frequency band; BRSHF, baroreflex slopes in the high-frequency band; BRSPS, baroreflex slopes, up-sloping segments; BRSNS, baroreflex slopes, down-sloping segments.

band. Similarly, there was no association for low- or high-frequency oscillations of systolic blood pressure, the second factor we analysed.

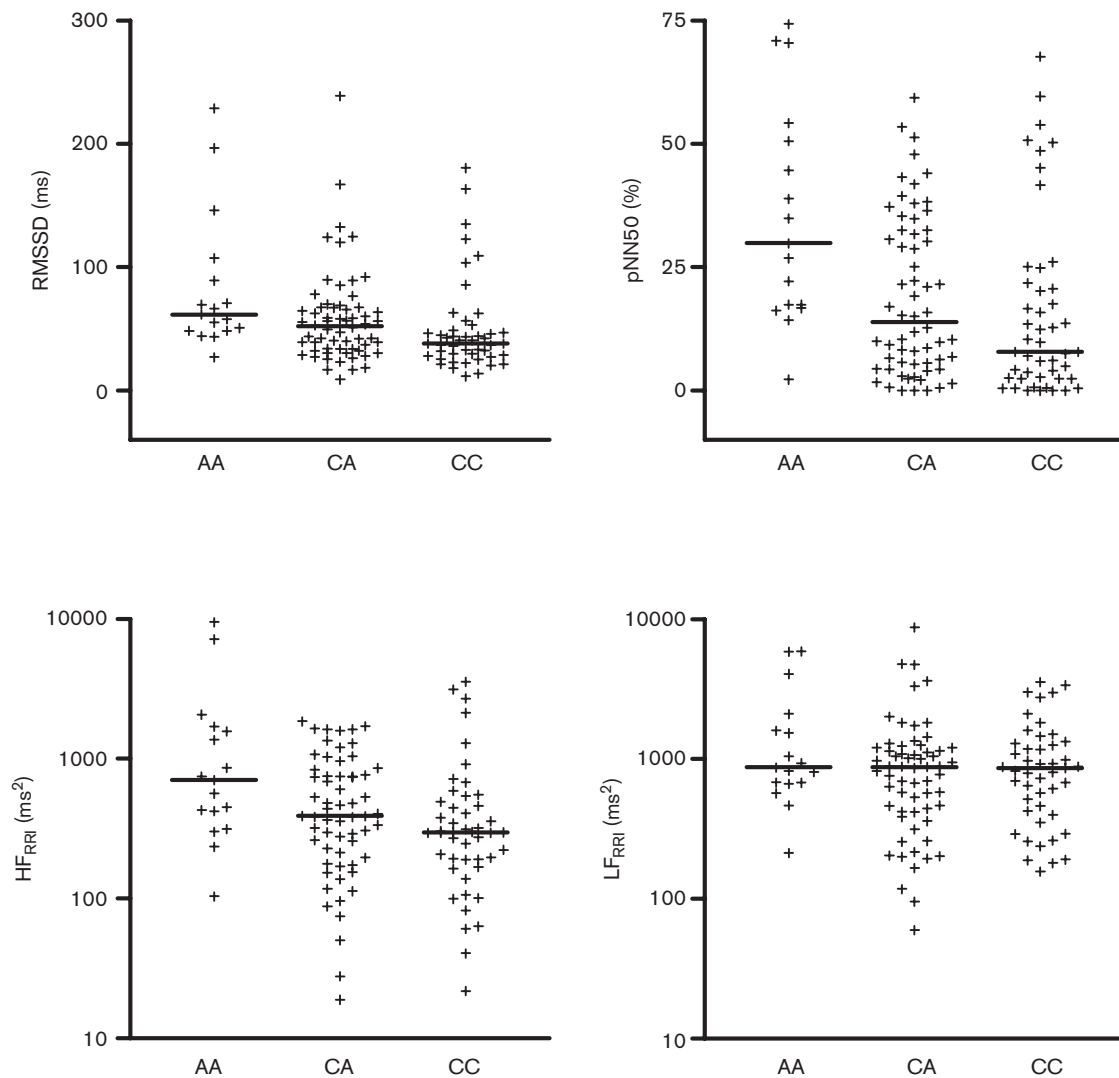
To show the individual data, we prepared Figure 1 which shows heart rate variability data. Homozygous exon 4b AA had higher heart rate variability, compared to CA or CC, particularly in the high-frequency range. The low-frequency range showed no association. Figure 2 shows baroreflex slope +, baroreflex slope in the high-frequency range and in the low-frequency range. Consistent with the heart rate variability data, homozygous AA persons had greater baroreflex slopes than CA or CC persons in the high-frequency range. In the low-frequency range, this association could not be shown. However, the variability of the measurements is considerable and larger numbers may be necessary to address this issue with greater precision.

## Discussion

The important finding in our study was the demonstration of associations between several SNPs within the  $\beta 1$  subunit gene and parameters relevant to baroreflex activity, particularly parasympathetic tone. To our knowledge, this study is the first to address directly possible molecular genetic mechanisms of baroreflex activity in normal subjects. The association data were fairly robust across various physiological measures. In this study, we relied on the maximum likelihood sib-pair analysis to avoid the confounding effect of population stratification, and report the results of the ANOVA for comparative reasons. However, the results with both methods were similar.

The association between several SNPs and RMSSD as well as pNN50 strongly suggests an important effect of the BK  $\beta 1$  subunit on autonomic control of heart rate. These measures of heart rate do not distinguish between different frequencies of heart rate variability. The high-frequency component of RR variability is strongly influenced by parasympathetic activity. The low-frequency component is influenced mainly by the sympathetic nervous system and to a lesser degree by the parasympathetic nervous system. The association with RR variability in the high-frequency band, but not in the low-frequency band, suggests that the genetic heterogeneity of BK  $\beta 1$  mainly influences rapid baroreflex-mediated adjustment of heart rate by the parasympathetic nervous system. The mouse models with a disrupted BK  $\beta 1$  subunit were both hypertensive and therefore had reset the baroreceptor response [2,3]. Whether or not the mice exhibited findings similar to those reported here is not known for certain. The mouse has a heart rate of 500–600 bpm and a respiratory rate between 80–100 breaths/min. Thus, the animal presents a special challenge for the kinds of measurements made in our human subjects. Neverthe-

Fig. 1



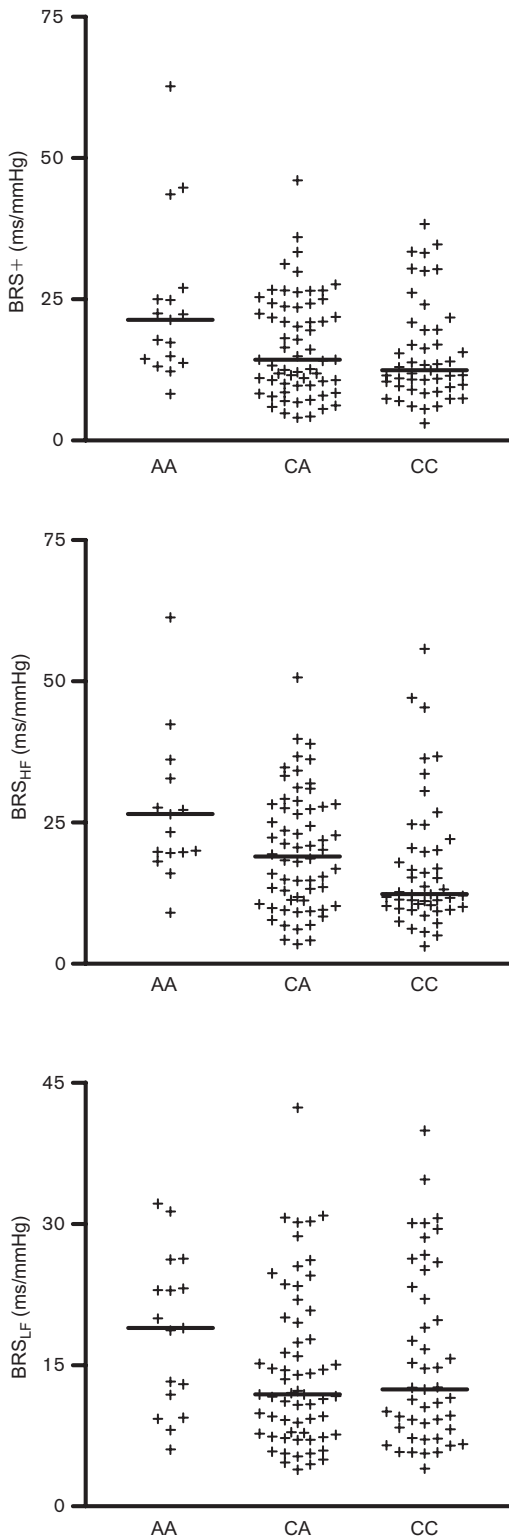
Heart rate variability is given in association with the single-nucleotide polymorphisms (SNPs) in exon 4. Persons homozygous AA had higher heart rate variability, compared to CA or CC, in particular in the high-frequency range. The low-frequency range showed no association. RMSSD, square root of the mean squared differences of successive normal-to-normal intervals; pNN50, proportion of successive normal-to-normal intervals differences greater than 50 ms; HF<sub>RRI</sub>, high-frequency R-R interval; LF<sub>RRI</sub>, low-frequency R-R interval.

less, with telemetric techniques and computer programs currently available, such analyses are feasible in the mouse (data not shown).

On the basis of our findings, we can only speculate where the BK channel site of action responsible for our responses might be located. BK channels have a wide distribution that extends far beyond the vasculature. They have been targeted in the central nervous system to protect organisms from neurological defects following stroke [15] and they play an important role in the function of sympathetic neurons, particularly in the generation of after-hyperpolarizations [16,17]. BK channels are important in the nitric oxide-mediated regula-

tion of adrenal catecholamine secretion [18]. Potassium channels are pivotal to neurotransmission, both at presynaptic and postsynaptic sites, where they influence both excitatory and inhibitory neurotransmission. BK channels were shown to have an inhibitory effect in sensory nerves [19]. A role for BK channel influences on sensory afferents in our study is also possible. Pedarzani *et al.* [20] relied on *in situ* hybridization analysis and documented the presence of 'big'-conductance  $\text{Ca}^{2+}$ - and voltage-activated  $\text{K}^{+}$  (BK) channel  $\alpha$ -subunit mRNA at very high levels in rat dorsal vagal neurons. Their observations may have relevance to our findings that  $\beta 1$  subunit variants are associated with the degree of vagal baroreflex tone.

Fig. 2



Baroreflex slope data were consistent with the heart rate variability data. Homozygous AA persons had greater baroreflex slopes than CA or CC persons in the high-frequency range. In the low-frequency range, this association could not be shown. BRS+, baroreflex slope +, BRS<sub>HF</sub>, baroreflex slope in the high-frequency range; BRS<sub>LF</sub>, baroreflex slope in the low-frequency range.

Channel genes are attractive candidate genes for baroreceptor function. Touch sensation and mechanoreceptor mechanisms have been linked to channels. For instance, genetic analyses of touch sensation and locomotion in *Caenorhabditis elegans* have implicated a new class of ion channels, the degenerins (DEG) in nematode mechanotransduction [21]. Related fly and vertebrate proteins, the epithelial sodium channel (ENaC) family, have been implicated in several important processes, including transduction of mechanical stimuli, pain sensation, gametogenesis, sodium reabsorption and blood pressure regulation. Drummond *et al.* [22] have tested members of the DEG/ENaC family that may be components of the baroreceptor mechanosensor. They found that the  $\gamma$  subunit of ENaC is localized to the site of mechanotransduction in baroreceptor nerve terminals innervating the aortic arch and carotid sinus [23]. Recently, Price *et al.* [24] identified the brain sodium channel (BNC1) as essential for the normal detection of light touch. The investigators indicated that BNC1 may be a central component of a mechanosensory complex. The human homologue of this gene would also be of considerable interest in terms of a candidate for human baroreflex function.

In summary, an analysis of six SNPs in the KCNMB1 gene supported a role for the subunit in cardiovascular regulation, since several important baroreflex regulatory mechanisms were associated with variants in the gene. Our findings may be of clinical relevance since patients with impaired baroreflex function suffer an increase in cardiovascular morbidity and mortality [25]. Finally, as supported by the mouse models [2,3], our findings could have a bearing on the propensity to develop hypertension. We studied normal subjects and since we found no direct association with blood pressure *per se*, that possibility remains speculative.

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