

AKAP10 (I646V) functional polymorphism predicts heart rate and heart rate variability in apparently healthy, middle-aged European-Americans

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Abstract

Previous evidence suggests that the dual-specific A kinase-anchoring protein 2 functional polymorphism (AKAP10 (A/G) I646V) influences heart rate (HR) and heart rate variability (HRV) in mice and humans ($N = 122$) with cardiovascular disease. Here, we asked whether this AKAP10 variant predicts HR and HRV in a large sample of healthy humans. Resting HR and short-term time and frequency domain measures of HRV (5 min during paced and unpaced respiration conditions) were assessed in a U.S. community sample ($N = 1,033$) of generally healthy men and women (age 30–54) of European ancestry. Each person was genotyped for the AKAP10 variant. As with previous work, the AKAP10 Val allele predicted greater resting HR (Paced $p < .01$; Unpaced $p < .03$) and diminished HRV (Paced $ps < .05$) suggesting that this variant may modulate the sensitivity of cardiac pacemaker cells to autonomic inputs, possibly conferring risk for arrhythmias and sudden cardiac death.

Descriptors: AKAP10, Heart rate variability, A kinase-anchoring protein 2, Autonomic function

Cardiovascular disease is a leading cause of death in the United States, with sudden cardiac death (SCD) taking an estimated 300,000 to 460,000 adult lives each year (de Vreede-Swagemakers et al., 1997; American Heart Association, 2007; Zheng et al., 2001). Unfortunately, SCD is often the first sign of cardiac illness (Weaver & Peberdy, 2002), hence posing a significant challenge for researchers to identify premorbid indicators of SCD. The heritable contribution to cardiovascular disease and SCD is complex (Nabel, 2003). Such complexity has led researchers to investigate genes regulating pathophysiologic pathways thought to enhance susceptibility to these conditions (e.g., autonomic cardiac dysregulation). Higher resting heart rate (HR) and lower tonic measures of autonomic-cardiac function (e.g., heart rate variability or HRV), are significant predictors of SCD related end points in apparently healthy individuals (Jouven et al., 2005; Levine, 1997; Liao et al., 1997; Tsuji et al., 1996). Autonomic nervous system dysregulation, particularly low parasympathetic

(vagal/cholinergic) activity, is also associated with psychosocial risk factors for coronary heart disease (e.g., hostility and depression), coronary atherosclerosis, and risk of clinical cardiac events (e.g., Tsuji et al., 1996; Ponikowski et al., 1997; Agelink et al., 2002; Carney, Freedland, & Veith, 2005; Rozanski, Blumenthal, & Kaplan, 1999). Biometric family and twin studies report significant genetic influence on parasympathetic HRV phenotypes with heritability estimates of up to 65% (Busjahn et al., 1998; de Geus et al., 2007; Kupper et al., 2004; Kupper et al., 2005; Riese et al., 2007; Singh et al., 2001; Sinnreich et al., 1999; Snieder et al., 1997; Snieder et al., 2007; Uusitalo et al., 2007).

A large-scale, age-stratified association study comparing 6,500 single nucleotide polymorphisms (SNPs) located in about 5,000 genes discovered that a SNP resulting in an amino acid change from Ile to Val at position 646 in the dual-specific A kinase-anchoring protein 2 (AKAP10, also called d-AKAP2) gene was associated significantly with a negative health prognosis in the older versus younger sample of humans which may be due, at least in part, to cardiac dysfunction (Kammerer et al., 2003). In this regard, the minor Val variant had a higher affinity to protein kinase A and greater atrioventricular node conduction than the Ile variant (Kammerer et al., 2003). Very recent molecular work suggests that this AKAP10 (I646V) genetic variant

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is associated with markers that predict an increased risk of SCD (Tingley et al., 2007). Specifically, in mouse embryonic stem cells, heterozygous gene trap disruption of the AKAP10 gene increases the responses of cultured cardiac cells in reaction to cholinergic signals (determined via cells treated with cholinergic and adrenergic agonists). Similar results for HR and square root of the mean squared differences of successive normal-to-normal interbeat intervals (RMSSD) were found in both heterozygous and homozygous mutant mice derived from these mouse embryonic stem cells, suggesting a dominant effect of the AKAP10 gene trap allele. The mutant mice eventually had cardiac arrhythmias and died prematurely. Additionally, a common variant of AKAP10 (646V, 40% of alleles) in a small sample of humans ($N = 122$) with known coronary heart disease was associated with increased resting HR and decreased overall HRV (i.e., standard deviation of normal-to-normal interbeat intervals or SDNN) (Tingley et al., 2007). These findings suggest that AKAP10 regulates HR by modulating the sensitivity of cardiac pacemaker cells to cholinergic vagal inputs in humans, possibly conferring risk for SCD.

Although the previous evidence suggests that AKAP10 I646V influences cholinergic-autonomic activity in mice and in a small sample of humans with established cardiovascular disease, it is not yet known whether this genetic variant is related to cholinergic-autonomic function in a healthy (no apparent cardiovascular disease) large community sample of European ancestry. In the present study, we aimed to investigate whether the same AKAP10 (I646V) genetic variant is related to both resting HR and time and frequency domain HRV phenotypes in a larger ($N = 1033$), more representative U.S. community sample of generally healthy, middle-aged men and women of European ancestry.

Method

Participants

Subjects were 1,033 Caucasians of European ancestry aged 30–54 ($M = 44.5 \pm 6.8$ years); 49% were men and none had a self-reported history of myocardial infarction or coronary revascularization, chronic kidney or liver disease, cancer, or neurological disorders or current use of psychotropic medications. Participants taking glucocorticoid and antihypertensive medications were excluded from the analyses. Participants were derived from a study of cardiovascular disease risk factor covariation in a community sample recruited by mass-mail solicitation from southwestern Pennsylvania, USA (principally Allegheny County). European ancestry was inspected by recording each participant's parents' and grandparents' country of birth and the participant's self-reported identity of their race. Informed consent was acquired in compliance with the guidelines of the University of Pittsburgh Institutional Review Boards. The investigation also conforms with the principals outlined in the Declaration of Helsinki.

Measures and Procedures

DNA Extraction and Analysis. Blood samples were collected in 10mM EDTA, and DNA was isolated from lymphocytes using a salting out procedure (Miller, Dykes, & Polesky, 1988). Genotyping of the AKAP10 (A/G) SNP (rs205462; A = Ile; G = Val) was achieved through polymerase chain reaction amplification and allele specific detection by fluorescence polarization

(Chen, Levine, & Kwok, 1999). Amplification used primers F: 5'-TATTTCTTTAGGCCAGGAAG-3' and R: 5'-GTAATCCCACAGCAGTTAATC-3'. The FP-TDI primer, 5'-GGAAGATTGCTAAAATGATAGTCAGTGAC-3', was used for detection. DNA amplification and genotyping was successful on 991 participants. The more common allele is labeled "A" and the less common allele is designated "G."

Heart Rate Variability. Participants were asked to refrain from caffeine for 4 h, alcohol and exercise for 12 h, and over-the-counter medications for 24 h prior to their measurement session. Participants were seated in a temperature-controlled recording chamber at the Behavioral Physiology Laboratory, University of Pittsburgh. Heart rate was recorded continuously by a standard, 2-lead electrocardiogram attached bilaterally to the wrists during an Unpaced and Paced respiration (each for 5 min). During the Paced period, participants were instructed to inhale and exhale naturally according to two auditory signals with a breathing rate of 11 breaths per min. Pacing respiration across participants was done to reduce variation in HRV estimates that may derive from between and within subject differences in respiration (Berntson et al., 1997; Ritz & Dahme, 2006). Electrocardiogram signals were amplified and filtered by Grass bioamplifiers and were acquired continuously, employing computerized analog to digital conversion at a rate of 1,000 samples per s (Debski et al., 1991). Respiration rate was assessed concurrently by thoracic strain-gauge.

Mean HR (beats per min) was obtained from the mean interbeat interval (IBI in ms) ($HR = 60,000/IBI$). Time domain analyses on the interbeat interval data were performed to ascertain the square root of the mean squared differences of successive normal-to-normal interbeat intervals (RMSSD) and the standard deviation of all normal-to-normal interbeat intervals (SDNN), commonly used measures of interval differences with RMSSD estimating high frequency variations in HR and SDNN estimating overall HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Frequency domain analyses were also performed to estimate HRV utilizing a program for spectral analysis of point events including a test for stationarity (Weber, Molenaar, & van der Molen, 1988). Spectral power analyses were performed on IBI data to estimate high frequency (HF) power (centered at each participant's respiration frequency (Hz) ± 0.015) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Pagani, Rimoldi, & Malliani, 1992). The HF power band thus shifts between subjects and conditions—it is the variability in heart period within .03 range of a person's breathing frequency. HF-HRV primarily reflects respiratory modulated parasympathetic (cholinergic) influences on heart rhythm (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Kleiger, Bigger, & Moss, 1997; Malliani et al., 1990). Since HF-HRV distributions were positively skewed, natural log transformations were performed to normalize these data before statistical analysis. Data of 56 participants were missing due to cardiac ectopy, equipment malfunction, or noisy ECG signal, leaving a total of 977 participants for the final analysis of the relation of AKAP10 genotype to HRV variables.

Control Measures. Heart rate variability has been shown to vary as a function of sex, age, body mass, and several lifestyle

Table 1. Sample Characteristics

Characteristic	N = 1,033
Sex (No. men:women)	503:530
Age (years: M ± SD)	44.5 ± 6.8
Education (years: M ± SD)	16.0 ± 2.8
Body Mass Index (kg/m ² : M ± SD)	26.8 ± 5.2
Smokers (%)	16
Physical Activity (KCAL during 7 days: M ± SD)	2518 ± 1795
Alcohol Intake (No. drinks during past month: M ± SD)	15.4 ± 31.5
Fasting Glucose Levels (mmol/l: M ± SD)	5.28 ± .81

behaviors (i.e., heavy alcohol consumption, physical activity, and smoking) (e.g., (Colhoun et al., 2001; Fagard, Pardaens, & Staessen, 1999; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Umetani et al., 1998). Additionally, several investigations have shown significant relations of impaired glucose metabolism to higher HR and lower HRV measures (Liao et al., 1995; Singh et al., 2000). Therefore, age, body mass index, self-reported smoking status (never smoked or ex-smoker vs. smoker), physical activity level [as measured by the Paffenbarger Physical Activity Questionnaire: estimates kilocalorie expenditure (Total KCAL) over a 7-day period was based on average blocks walked, stairs climbed, and leisure time activity during the past year] (Paffenbarger, Wing, & Hyde, 1978), and alcohol consumption during the past month (number of alcoholic beverages with 1 drink = 12 oz. beer, 4–5 oz. wine, and 1.5 oz. of liquor) were measured as control factors. Fasting glucose levels were also considered a control factor and were assayed by standard colorimetry by the Heinz Nutrition Laboratory, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, which has met the criteria of the Centers for Disease Control since 1982.

Statistical Analyses

All analyses were performed using SPSS for Windows (Version 15). Gene frequencies were estimated by gene counting, and genotypes were tested for fit to Hardy-Weinberg equilibrium by Chi-square analysis. Correlations were computed for covariates and HRV measures. To evaluate the hypothesis that AKAP10 G allele carriers have higher HR (as measured by mean IBI) and diminished RMSSD, SDNN, and HF-HRV, separate hierarchical multiple regression analyses were performed with AKAP10 genotypes (dummy coded) predicting each of these cholinergic criterion variables controlling for sex, age, BMI, smoking status, physical activity level, average number of alcoholic beverages consumed per month, and fasting glucose levels in step 1.

Results

Gene frequencies of the A and G alleles were 0.60 and 0.40, respectively. The resulting distribution of genotypes (AA = 349, AG = 489, and GG = 153) conformed to Hardy-Weinberg equilibrium ($\chi^2 = 0.77$, n.s.) and did not differ between men and women ($\chi^2 = 3.33$, 0.19). Sample characteristics are provided in Table 1. Several significant correlations were found between covariates and HRV measures except for smoking status (see Table 2). All of the correlations were in the expected direction.

As expected, significant and direct Pearson Product Moment correlations were found between IBI and the HRV measures for the unpaced respiration condition (SDNN: $r = .45$; RMSSD: $r = .52$; HF-HRV: $r = .14$; all $ps < .001$) and the paced respiration condition (SDNN: $r = .45$, $p < .001$; RMSSD: $r = .52$, $p < .001$; HF-HRV: $r = .06$, $p < .05$). Highly significant and direct correlations were also noted between the time domain HRV measures (RMSSD and SDNN) for the unpaced respiration condition ($r = .85$, $p < .0001$) and paced respiration condition ($r = .91$, $p < .0001$). The frequency domain HF-HRV associa-

Table 2. Correlations Among Control Variables and Interbeat Interval (IBI) and Heart Rate Variability (HRV) Estimates During Unpaced and Paced Respiration Conditions

	Sex ^S		BMI ^P kg/m ²	Smoking Status ^S		Alcohol Intake ^P Drinks/month	Physical Activity ^P KCAL	Glucose ^P mmol/l
	0 = Male 1 = Female	Age ^P years		1 = non/ex smoker 2 = current user				
	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	<i>r</i> (<i>p</i>)	
Unpaced IBI (ms)	-.082 (.01)	.031 (n.s.)	-.152 (.0001)	-.004 (n.s.)	.135 (.0001)	.229 (.0001)	-.084 (.009)	
Unpaced RMSSD (ms)	.151 (.0001)	-.302 (.0001)	-.099 (.002)	.001 (n.s.)	.028 (n.s.)	.122 (.0001)	-.127 (.0001)	
Unpaced SDNN (ms)	-.005 (n.s.)	-.331 (.0001)	-.069 (.018)	-.015 (n.s.)	-.012 (n.s.)	.113 (.0001)	-.114 (.0001)	
Unpaced HF-HRV (ln)	.275 (.0001)	-.352 (.0001)	-.098 (.002)	-.020 (n.s.)	-.032 (n.s.)	.021 (n.s.)	-.160 (.0001)	
Paced IBI (ms)	-.088 (.006)	.038 (.24)	-.096 (.003)	.006 (n.s.)	.123 (.0001)	.197 (.0001)	-.068 (.04)	
Paced RMSSD (ms)	.185 (.0001)	-.263 (.0001)	-.098 (.002)	-.010 (n.s.)	.000 (n.s.)	.114 (.0001)	-.127 (.0001)	
Paced SDNN (ms)	.01 (n.s.)	-.282 (.0001)	-.056 (.047)	-.007 (n.s.)	-.045 (.085)	.124 (.0001)	-.129 (.0001)	
Paced HF-HRV (ln)	.325 (.0001)	-.410 (.0001)	-.146 (.0001)	-.022 (n.s.)	-.094 (.003)	.05 (n.s.)	-.187 (.0001)	

Note: Pearson's Product Moment correlation^P coefficients are shown for continuous data, and Spearman's rho correlations^S are depicted for non-continuous data. IBI = Interbeat interval; RMSSD = Mean squared differences of successive normal-to-normal interbeat intervals; HF-HRV = High frequency heart rate variability.

Table 3. Pearson Product-Moment Correlations Among Mean Interbeat Interval (IBI) and Heart Rate Variability (HRV) Measures [square root of the mean squared differences of successive normal-to-normal interbeat intervals (RMSSD), standard deviation of all normal-to-normal interbeat intervals (SDNN), and high frequency (HF)-HRV] During Paced and Unpaced Respiration Conditions (all p s < .003)

	Unpaced IBI (ms)	Unpaced SDNN (ms)	Unpaced RMSSD (ms)	Unpaced HF-HRV (ln)
Paced IBI (ms)	.91	.38	.44	.10
Paced SDNN (ms)	.50	.73	.78	.46
Paced RMSSD (ms)	.53	.72	.86	.49
Paced HF-HRV (ln)	.12	.51	.57	.67

tions with the time domain measures were significant and positive, but of a moderate magnitude for both respiration conditions (Unpaced: RMSSD, $r = .59$ and SDNN, $r = .55$; Paced: RMSSD, $r = .61$ and SDNN, $r = .59$). Pearson Product Moment correlations among these HR and HRV measures between the two respiration conditions are displayed in Table 3.

AKAP10 genotype (AA, AG, GG) predicted mean IBI (Unpaced: $\beta = -.08$, $p < .01$; Paced: $\beta = -.07$, $p < .03$) (see Table 4). As compared to AA homozygotes, participants having any G (Val) allele displayed a shorter mean IBI (or higher mean HR), with GG homozygotes showing the shortest mean IBI (or highest mean HR) (see Figure 1a and b). AKAP10 genotype (AA, AG, GG) also significantly predicted mean SDNN (Paced: $\beta = -.063$, $p < .045$) (see Table 4). Participants carrying a G (Val) allele showed lower HRV than AA homozygotes (see Figure 2). AKAP10 genotype (AA, AG, GG) significantly predicted mean RMSSD during the paced respiration period ($\beta = -.06$, $p < .05$) with a trend noted for unpaced respiration condition ($\beta = -.059$, $p < .059$) (see Table 4). Participants carrying a G (Val) allele showed lower HRV than AA homozygotes (see Figure 3a and b). No significant relations or trends of AKAP10 genotype on the HF-HRV components were observed.

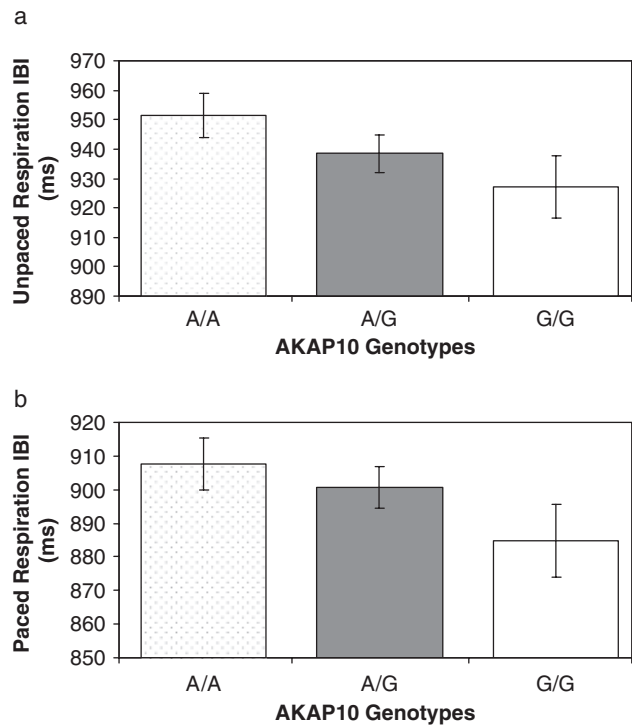


Figure 1. AKAP10 (A/G) SNP genotype on mean interbeat interval (IBI) during unpaced (1) and paced (2) respiration conditions with bidirectional standard error bars.

Discussion

In this report, we present evidence consistent with prior work (Tingley et al., 2007; Kammerer et al., 2003) that polymorphic variation in the dual-specific A kinase-anchoring protein 2 (AKAP10) gene is significantly associated with HR and HRV, which are significant risk factors for sudden cardiac death (SCD). Middle-aged adults (both men and women) who possess an AKAP10 genotype containing a G (Val) allele exhibited shorter interbeat intervals or greater resting HR and lower HRV than subjects homozygous for the alternate A allele, independent of

Table 4. Results of Regression Models Examining AKAP10 (A/G) Genotype as Predictor of Mean Interbeat Interval (IBI), Standard Deviation of Normal-to-Normal Interbeat Intervals (SDNN) and Mean Squared Differences of Successive Normal-to-Normal Interbeat Intervals (RMSSD) During Unpaced and Paced Respiration Conditions with Standard Covariates Entered in Step 1 and AKAP10 Genotypes Entered into Step 2

Predictor	Unpaced IBI			Unpaced RMSSD			Paced IBI			Paced RMSSD			Paced SDNN		
	Beta	p	Adj R ²	Beta	p	Adj R ²	Beta	p	Adj R ²	Beta	p	Adj R ²	Beta	p	Adj R ²
Step 1:			.078			.119			.058			.094			.097
Sex 0 = male, 1 = female	-.125	.000		.117	.000		-.123	.000		.094	.005		.001	.966	
Age	.048	.132		-.296	.000		.057	.078		-.252	.000		-.268	.000	
Body Mass Index	-.116	.001		-.047	.148		-.064	.057		-.044	.189		-.024	.462	
Smoking Status 1 = non/ex smoker 2 = current user	-.005	.873		.050	.122		-.000	.996		.036	.275		.019	.553	
Alcohol Intake	.000	.986		-.030	.364		.000	.977		-.031	.355		-.035	.291	
Physical Activity	.209	.000		.090	.005		.191	.000		.108	.001		.109	.001	
Glucose	-.081	.017		-.048	.146		-.079	.021		-.058	.085		-.081	.015	
Step 2:			.084			.121			.061			.097			.10
AKAP10 - 1 = AA, 0 = AG, 1 = GG	-.08	.01		-.059	.059		-.070	.03		-.06	.05		-.063	.045	

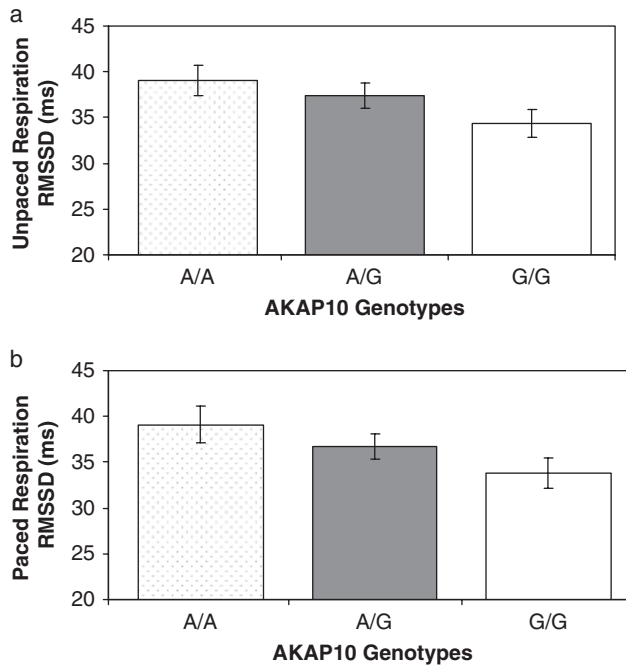


Figure 2. AKAP10 (A/G) SNP genotype on mean squared differences of successive normal-to-normal interbeat intervals (RMSSD) during unpaced (1) and paced (2) respiration conditions with bidirectional standard error bars.

sex, age, smoking status, alcohol consumption, physical activity level, and fasting glucose levels. Taken together with the results of previous work (Kammerer et al., 2003; Tingley et al., 2007), these findings suggest that this AKAP10 (I646V) variant may

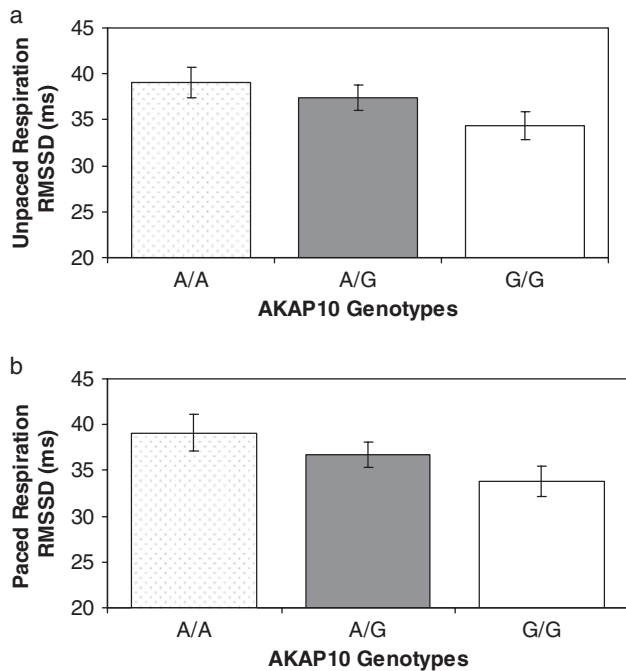


Figure 3. AKAP10 (A/G) SNP genotype on mean squared differences of successive normal-to-normal interbeat intervals (RMSSD) during unpaced (a) and paced (b) respiration conditions with bidirectional standard error bars.

modulate the sensitivity of cardiac pacemaker cells to sympathetic-vagal interactions at the sinus node in humans, possibly conferring risk for arrhythmias and SCD.

Our results are concordant with previous work (Kammerer et al., 2003; Nishihama et al., 2007; Tingley et al., 2007) showing that the G (Val) allele is related to higher HR and diminished measures of HRV in a community sample of men and women of European ancestry and extend prior observations to a much larger sample of participants without clinically apparent atherosclerotic cardiovascular disease. The variance accounted for by these relations is small, but consistent with the understanding that HR and HRV are complex traits that are likely influenced by multiple genes of small effect as well as gene by gene and gene by environment interactions. In this regard, other genetic variants of different causal pathways found to influence or be influenced by HR and HRV, such as nitric oxide (eNOS (T-786C), GCH1 (C+243T), and NOS1AP four SNPs) (Binkley et al., 2005; Zhang et al., 2007; Newton-Cheh et al., 2007, respectively) and angiotensin-converting enzyme (ACE insertion/deletion polymorphism in intron 16) pathways (Thayer et al., 2003), together with AKAP10 (I646V) may have additive or synergistic effects that yield significant clinical information.

Contrary to our hypothesis, HF-HRV did not yield significant relations with the AKAP10 variant studied here. This may be due to several reasons. First, it is hypothesized here that, due to our pattern of findings, this AKAP10 variant may have more influence on basal HR and sympathetic-vagal interactions at the sinus node (*accentuated antagonism* (Levy, 1971)) (as indexed by RMSSD and SDNN) rather than variability in HR that is modulated by only vagal tone associated with breathing as estimated by HF-HRV. In support of this, RMSSD has been shown to be more highly correlated with HR than HF-HRV and may reflect some sympathetic influences (Berntson, Lozano, & Chen, 2005) and in our own data shown in Table 3. Some previous research has also shown that RMSSD not only measures high frequency fluctuations from parasympathetic influences, but also may measure low frequency fluctuations derived from sympathetic influences (Berntson et al., 2005). The same study reported that RMSSD was strongly influenced by tonic heart period as well. Another study demonstrated RMSSD to be less sensitive to respiration than HF-HRV (Penttila et al., 2001). Second, while preprocessing methods were carefully conducted with the frequency domain measures in the current study, frequency domain measures have been shown to be estimated with more bias and variability than time domain measures; frequency domain measures should therefore be interpreted with caution (Kuss et al., 2008; Berntson et al., 1997). Third, it has been clearly shown that both respiration rate and tidal volume can affect HF-HRV (Ritz & Dahme, 2006). Although care was taken in the current study to control for respiration in our HRV measurements, in future work, it may be beneficial to also control for tidal volume as well as respiration in testing similar hypotheses in order to better understand, without this potentially confounding factor, autonomic mechanisms that may be influenced by this AKAP10 variant.

If these AKAP10 findings are corroborated in other independent samples, these findings should contribute to our understanding of autonomic modulation of heart rate and possibly the pathogenic processes involved in risk for SCD in the general population. In a previous epidemiological study, the AKAP10 (I649V) allele has been associated with an increased risk of myocardial infarction (MI) due to coronary artery disease (Nishihama et al., 2007). This study of unrelated Japanese individuals

(1,192 subjects having an MI and 2,291 controls) showed a higher proportion of Val allele present in those having an MI (Nishihama et al., 2007). Although too early to speculate here, it may be worthwhile to explore the potential mechanistic relation between this AKAP10 variant and HRV to coronary artery disease as efferent cholinergic activity of the vagus nerve has been shown to inhibit cellular activation of macrophages and inflammatory cytokines, which have been implicated in atherosclerosis (Tracey, 2002). In the future, it may also prove beneficial to evaluate inflammatory markers in relation to this AKAP10 (I646V) variant to further understand the relationship of this Val allele with SCD and coronary artery disease. In addition, this relation may be pertinent to depression phenotypes, in which disruption of the cholinergic system has been posited, and to the augmented risk of coronary disease events that is predicted by low vagal activity (e.g., Tsuji et al., 1996; Ponikowski et al., 1997; Kleiger et al., 1997; Grossman, Brinkman, & deVries, 1992; Agelink et al., 2002; Carney, Freedland, & Veith, 2005; Rozan-

ski, Blumenthal, & Kaplan, 1999). Future work could also investigate AKAP10 variation in association with other cardiovascular disease risk factors, such as behaviorally-evoked cardiovascular reactivity and recovery, baroreceptor sensitivity, and blood pressure variability.

Although our results concur with previous molecular work (Tingley et al., 2007), the present findings should be interpreted cautiously, since they are based on simple association analysis. As in all investigations that involve samples of unrelated individuals, spurious genetic association derived from unknown sources of population substructure remains a theoretical possibility (Burmeister, 2007; Malhotra & Goldman, 1999). Confirmatory studies are warranted using more definitive genetic methodologies, such as family-based association designs (Allison, 1997; Ewens & Spielman, 1995) or statistical adjustment for population stratification by concurrent evaluation of multiple single-nucleotide polymorphisms (Devlin, Roeder, & Wasserman, 2000).

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