Left Ventricular Radial Function Associated With Genetic Variation in the cGMP-Dependent Protein Kinase

Tatiana Kuznetsova, Lorena Citterio, Laura Zagato, Simona Delli Carpini, Lutgarde Thijs, Nunzia Casamassima, Jan D’hooge, Giuseppe Bianchi, Paolo Manunta, Jan A. Staessen

Abstract—cGMP-dependent protein kinase type I is a major mediator of cGMP signaling in the cardiovascular system. Recent studies on cardiac-specific PRKG1 knockout mice demonstrated that cGMP-dependent protein kinase type I mediates the negative inotropic effect of cGMP in the myocardium. We therefore investigated the association between left ventricular (LV) function and common polymorphisms in the PRKG1 gene in a general population. In 609 randomly selected participants (51.2% women; mean age, 48.8 years; 36.6% hypertensive) who were free from overt cardiac disease, we performed echocardiography and genotyped intronic tag single-nucleotide polymorphisms (SNPs) rs1904694, rs7897633, and rs7905063 in PRKG1. On the basis of color Doppler myocardial motion data, we calculated end-systolic longitudinal and radial deformation (strain) of the LV inferolateral wall. In multivariable-adjusted analyses accounting for confounders and relatedness, systolic radial strain was significantly (P≤0.005) higher in homozygotes for rs1904694 (GG), rs7897633 (AA), and rs7905063 (TT) compared with heterozygotes or noncarriers. Haplotype analysis confirmed that LV radial strain was significantly higher in GAT homozygotes than in noncarriers (62.3% versus 56.0%; P=0.0005). Transmission of the PRKG1 GAT haplotype to informative offspring was associated with higher LV radial strain (effect size, 6.11%; P=0.017). For other LV phenotypes, none of the phenotype-genotype associations reached statistical significance. In conclusion, LV systolic radial function was associated with common polymorphisms in PRKG1. If experimental studies and longitudinal follow-up of LV function confirm the causality of this association, interference with cGMP-dependent protein kinase type I function might be a target for pharmacological intervention. (Hypertension. 2013;62:1034-1039.)

Key Words: Doppler ultrasound imaging ■ echocardiography ■ left ventricular function ■ PRKG1 protein

Cyclic guanosine monophosphate (cGMP), an intracellular second messenger, exerts its action by cGMP-dependent protein kinases (PKG-1) and cGMP-regulated phosphodiesterases. In the cardiovascular system, cGMP signaling is an important regulator of endothelial cell, vascular smooth muscle, and cardiomyocyte function.1 Studies of isolated myocytes from wild-type and cardiac-specific PKG-1 knockout mice2 demonstrated that cGMP–PKG-1 signaling mediates a negative inotropic effect. Furthermore, in cardiac myocytes, adenosinal expression of PKG-1 attenuated hypertrophy via inhibition of calcineurin–nuclear factor of activated T-cells signaling.3 A recent genome-wide association study4 found that the common genetic variants (tag) in human PKG-1 gene (PRKG1) were significantly associated with changes in diastolic blood pressure in response to an acute salt load in 478 never-treated patients with hypertension.

Taken together, the above observations raise the possibility that genetic variability in PRKG1 might affect left ventricular (LV) function and structure. In the Flemish Study on Environment, Genetics, and Health Outcomes (FLEMENGHO), we therefore investigated whether LV function and structure were associated with tag PRKG1 polymorphisms. In this study, we assessed LV phenotypes by using classical M-mode and 2-dimensional echocardiography as well as tissue Doppler imaging, which is a sensitive technique to detect early stages of LV dysfunction.5,6

Methods

Study Participants

The Ethics Committee of the University of Leuven approved the FLEMENGHO study. As described in detail in previous publications,5,9 from August 1985 to December 2005, we recruited a random sample of families from a geographically defined area in northern Belgium. From May 2005 to April 2009, we reinvited 942 participants for a follow-up examination, including echocardiography, at our field center. We obtained informed written consent from 752 subjects (participation rate, 79.5%). From the current analysis, we excluded 67 subjects because of LV remodeling attributable to myocardial infarction or coronary revascularization (n=24), the presence of moderate or severe...
valvular abnormalities (n=32), atrial fibrillation (n=6), an artificial pacemaker (n=2), or frequent extrasystolia (n=3). We additionally discarded 70 subjects from analysis because the color Doppler myocardial images were of insufficient quality to assess LV strain patterns. The polymerase chain reaction did not yield a reliable genotype in 6 subjects. Thus, the number of participants statistically analyzed totaled 609.

Echocardiography

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for ≥3 hours before echocardiography.

Data Acquisition

One experienced physician (T.K.) did the ultrasound examination according to a standardized protocol as published elsewhere, using a Vivid 7 Pro (GE Vingmed, Horten, Norway) interfaced with a 2.5-MHz phased-array probe. With the subjects in partial left decubitus position and breathing normally, the observer obtained images from the parasternal long- and short-axis views and from the apical 4- and 2-chamber and 3-chamber views. M-mode echocardiograms of the LV were recorded from the parasternal long-axis view under control of the 2-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae. All recordings included ≥5 cardiac cycles and were digitally stored for off-line analysis.

Using tissue Doppler imaging, the observer recorded high-intensity myocardial velocity signals at a high frame rate (>190 frames per second) while adjusting the imaging angle to ensure parallel alignment of the ultrasound beam with the myocardial segment of interest. The Nyquist limit was set as low as possible to avoid aliasing.

Off-Line Analysis

The same observer analyzed the recorded images, averaging 3 heart cycles for statistical analysis, using a workstation running the EchoPac software, version BT11.0.0 (GE Vingmed, Horten, Norway). The postprocessing of echocardiograms was performed by an observer blinded to the genetic results. LV internal diameter and interventricular septal and posterior wall thickness were measured at end diastole from the 2-dimensionally guided M-mode tracing, as described in the American Society of Echocardiography guideline. End-diastolic LV dimensions were used to calculate LV mass by an anatomically validated formula. LV mass was indexed to body surface area. LV end-systolic and end-diastolic volumes were measured off-line using the standard biplane Simpson method.

We assessed LV diastolic function using recordings of conventional blood flow and tissue Doppler velocities. Pulsed-wave Doppler signals of transmitral blood flow were used to measure peak early (E) and late (A) diastolic velocities. From the pulsed-wave tissue Doppler imaging recordings, we measured the early (e') and late (a') peak diastolic velocities of the mitral annulus displacement and the e'/a' ratio at the 4 acquisition sites (septal, lateral, inferior, and posterior). We calculated the E/e' ratio by dividing transmitral E peak by e' averaged from the 4 acquisition sites.

To define myocardial deformation during systole, we extracted strain curves off-line from color tissue Doppler images, using dedicated software as previously described. The SPEQLE package (version 4.6.2) allows M-mode tracking of the myocardium to ensure that the sample volume is maintained in the same anatomic position throughout the cardiac cycle. We positioned the sampling volume in the basal portion of the interrogated wall at the level of the posterior chordae tendineae. To compute end-systolic strain, from now on referred to as strain, we averaged 3 consecutive cycles. We calculated the radial strain of the inferolateral wall and the longitudinal strain of the inferior and inferolateral walls by measuring the spatial velocity gradient over time in a sampling area of 5 mm and 10 mm, respectively. The beginning and ending of the ejection phase were determined from the simultaneously recorded ECG and the continuous-wave Doppler velocity trace at the level of the aortic valve. We used lateral averaging of 3 to 5 beams/pixel. Because there were no differences between the inferolateral and inferior walls in longitudinal strain, for statistical analysis, we averaged these measures and used their absolute values.

Other Measurements

At the examination center, trained study nurses administered a questionnaire to collect detailed information on each subject’s medical history, smoking and drinking habits, and intake of medications. Hypertension was defined as blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic (average of 5 consecutive readings at the examination center) or as the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters.

Statistical Methods

For database management and statistical analysis, we used SAS software, version 9.3 (SAS Institute, Cary, NC). We compared means and proportions using ANOVA and χ² test, respectively. We tested the Hardy–Weinberg equilibrium and linkage disequilibrium and reconstructed haplotypes using the PROC ALLELE and PROC HAPLOTYPE procedures implemented in the genetics module of the SAS software.

We performed both population- and family-based analyses. In the former approach, we tested the association of dependent variables (LV phenotypes) with the genotypes of interest by use of a mixed model. This technique allows accounting for covariables and the nonindependence of observations within families. We analyzed phenotype-genotype associations by applying codominant and recessive models. Covariables with known physiological relevance for LV structure and function were included as fixed effects, whereas family cluster was modeled as a random effect.

In the family-based analysis, we performed the transmission disequilibrium test for quantitative traits. We evaluated the within- and between-family components of phenotypic variance using the orthogonal model as implemented by Abecasis et al. in the QTDT software (version 2.6.1; http://www.sph.umich.edu/csg/abecasis/QTDT). In this model, only the between-family component is sensitive to population structure, whereas the within-family component is significant in the presence of transmission disequilibrium. We also calculated the heritability of LV radial strain in siblings using a variance components–based approach as implemented in the QTDT software.

Results

Characteristics of Participants

The 609 participants included 312 (51.2%) women and 223 (36.6%) patients with hypertension of whom 125 (20.5%) were on antihypertensive drug treatment. Mean age (±SD) was 48.8±14.4 years, and it ranged from 16 to 86 years. Table 1 lists the clinical and echocardiographic characteristics of the participants by sex. Compared with men, women had lower systolic and diastolic blood pressures, higher heart rate, and less frequently reported intake of alcohol (Table 1). Left atrium volume, LV diameter, wall thickness, and LV mass index (Table 1) were significantly greater in men than in women (P<0.0001), whereas the echocardiographic indexes reflecting systolic function, such as ejection fraction and radial strain, were greater in women than in men (Table 1).
### Table 1. Characteristics of Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women (n=312)</th>
<th>Men (n=297)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>49.4±14.2</td>
<td>48.2±14.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.0±6.9</td>
<td>175.6±7.0†</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.5±13.5</td>
<td>81.0±11.0†</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.8±4.6</td>
<td>26.3±3.3</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>125.7±17.1</td>
<td>129.4±14.3*</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>77.6±9.3</td>
<td>81.7±9.3†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>62.7±8.7</td>
<td>58.9±9.0†</td>
</tr>
<tr>
<td><strong>Questionnaire data, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>72 (23.1)</td>
<td>70 (23.6)</td>
</tr>
<tr>
<td>Drinking alcohol</td>
<td>75 (24.0)</td>
<td>181 (60.9)†</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>108 (34.6)</td>
<td>115 (38.7)</td>
</tr>
<tr>
<td>Treated for hypertension</td>
<td>67 (21.5)</td>
<td>58 (19.5)</td>
</tr>
<tr>
<td><strong>Echocardiographic measurements</strong></td>
<td></td>
<td></td>
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<tr>
<td>Conventional echocardiography</td>
<td></td>
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<tr>
<td>Left atrial volume index, mL/m²</td>
<td>21.0±5.1</td>
<td>23.2±5.9†</td>
</tr>
<tr>
<td>LV internal diameter, cm</td>
<td>4.08±0.38</td>
<td>5.25±0.41†</td>
</tr>
<tr>
<td>Interventricular septum, cm</td>
<td>0.92±0.15</td>
<td>1.03±0.17†</td>
</tr>
<tr>
<td>Posterior wall, cm</td>
<td>0.83±0.13</td>
<td>0.93±0.13†</td>
</tr>
<tr>
<td>LV mass index, g/m²</td>
<td>82.7±17.2</td>
<td>98.8±20.1†</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>69.7±7.1</td>
<td>68.0±6.7*</td>
</tr>
<tr>
<td>Diastolic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmirtal E/A ratio</td>
<td>1.30±0.44</td>
<td>1.35±0.53</td>
</tr>
<tr>
<td>TDI E' peak, cm/s</td>
<td>12.0±3.4</td>
<td>12.0±3.7</td>
</tr>
<tr>
<td>E/E' ratio</td>
<td>7.11±1.93</td>
<td>6.47±1.69†</td>
</tr>
<tr>
<td>TDI end-systolic strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal, %</td>
<td>22.6±3.6</td>
<td>22.1±3.6</td>
</tr>
<tr>
<td>Radial, %</td>
<td>59.0±12.6</td>
<td>56.4±11.6*</td>
</tr>
</tbody>
</table>

E/A indicates the ratio of the transmitral blood flow velocities in early (E) and late (A) diastole; E', the peak velocity of the mitral annulus in early diastole; and E/E', the ratio of the transmitral blood flow (E) and mitral annulus velocities (E') in early diastole.

Values are mean (±SD) or number of subjects (%). LV indicates left ventricle; and TDI, tissue Doppler imaging.

Significance of the sex difference:
*P<0.01.
†P<0.001.

### Table 2. Selected SNPs for PRKG1

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Chromosome Position</th>
<th>Location Type</th>
<th>Allele (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1904694</td>
<td>52905494</td>
<td>Intron</td>
<td>G (0.39)</td>
</tr>
<tr>
<td>rs7897633</td>
<td>52957721</td>
<td>Intron</td>
<td>A (0.52)</td>
</tr>
<tr>
<td>rs7905063</td>
<td>52964590</td>
<td>Intron</td>
<td>T (0.49)</td>
</tr>
</tbody>
</table>

SNP ID is a GenBank ID number (National Center for Biotechnology Information [NCBI]). Gene map position and SNP function were taken from the most recent human genome sequence assemblies (NCBI Build 37.3). Allele frequencies were calculated from the present population. ID indicates identification number; and SNP, single-nucleotide polymorphism.

### Population-Based Association Study

While accounting for family clusters and adjusting for important covariates, such as sex, age, body mass index, systolic blood pressure, and antihypertensive treatment (Table 3), the systolic radial strain was significantly higher in homozygotes for the coded allele of rs1904694 (GG), rs7897633 (AA), and rs7905063 (TT) than in noncarriers or heterozygotes (P≤0.0033). Haplotype analysis further confirmed that LV radial strain was significantly (P=0.0005) higher in GAT homozygotes of the PRKG1 gene (62.1%) than in heterozygotes (55.5%) and noncarriers (56.6%). We repeated our analyses after exclusion of subjects on antihypertensive drugs. In 484 untreated subjects, our findings remained consistent. The systolic radial strain in untreated subjects was significantly higher in homozygotes for the coded allele of studied SNPs than in noncarriers or heterozygotes (P≤0.013). For other LV phenotypes (Table 4), none of the phenotype–genotype associations reached statistical significance.

### Family-Based Association Study

Our total study population (n=609) included 399 founders from 175 families and 210 informative offspring belonging to 49 families (mean age, 34.9±9.9 years; 45.2% women). The number of offspring per informative family included in our family-based analyses amounted to 1 in 11 families, 2 in 12 families, 3 in 8 families, and >3 in 18 families. In the fully-adjusted model, LV radial strain showed moderate heritability (h²=0.32; P=0.025) in 210 offspring. We adjusted the QTDT analyses as described above. LV radial strain significantly increased only with the transmission of 2 rs1904694 G alleles (n=42) to offspring. The effect size of the within-family component averaged +7.97% (χ²=14.7; P=0.0006). On the contrary, for the A allele of rs7897633 (P=0.0036) and the T allele of rs7905063 (P=0.024), the between-family components of variation in LV radial strain were statistically significant, whereas the within-family components did not reach significance (P≥0.17). The transmission of 2 GAT alleles of the PRKG1 gene to informative offspring (n=23) was also significantly associated with higher LV radial strain (effect size, 6.11%; χ²=8.16; P=0.017).

### Discussion

The key findings of the present study were that the LV systolic radial strain was associated with genetic variation in the PRKG1 gene. LV radial strain was significantly higher in GAT homozygotes of the PRKG1 gene than in heterozygotes and noncarriers. The family-based analyses included only 210 informative offspring but confirmed that transmission of 2 rs1904694 G alleles...
or 2 GAT alleles to offspring was associated with higher LV radial strain. On the contrary, we did not observe any association of the genetic variants in PRKG1 gene and other LV phenotypes including LV mass and diastolic function.

To our knowledge, no previous study reported on the association of LV phenotypes with the PRKG1 gene. The PRKG1 gene product, protein kinase G-1 (PKG-1), is a serine/threonine-specific protein kinase that is activated by cGMP.\(^1\) PKG-1 regulates vascular smooth muscle relaxation and modulates the contractility, growth, and apoptosis of cardiomyocytes.\(^1,3,13\) In isolated myocytes from wild-type and cardiac-specific PKG-1 knockout mice,\(^2\) the cGMP–PKG-1 signaling mediated a negative inotropic effect. In this study, the cGMP analogues (8-Br-cGMP and 8-pCPT-cGMP) reduced the cardiomyocyte force of contraction by \(\approx\)30\% in electrically driven heart muscle from wild-type but not from PKG-1 knockout mice.\(^2\) This effect might be partially attributed to PKG-1 modulation of cardiomyocyte calcium responsiveness, such as reduction of L-type calcium current\(^1\) and phosphorylation of troponin I.\(^1,6,17\) The latter mechanism leads to less affinity of troponin C to calcium and, therefore, to a depressed contractility.\(^1,6,17\) Therefore, cGMP–PKG, countering cAMP stimulation, is considered an independently signaling system, which blunts cardiomyocyte contraction and growth while enhancing relaxation.\(^18\)

Involvement of the cGMP–PKG signaling pathway in cardiac contractility makes the PRKG1 gene a possible candidate for heart failure. In a genome-wide association study,\(^4\) common genetic variants in PRKG1 were significantly associated with the response of diastolic blood pressure to an acute salt load in 478 untreated patients with hypertension. The G, A, and T alleles were associated with significantly increased diastolic blood pressure (\(\Delta\) ranged from +1.87 to +2.06 mm Hg) after 120 minutes of salt load, and they might be therefore considered as risk alleles for salt sensitivity.\(^4\) It was suggested that the above-mentioned risk alleles influence the inhibitory effect on renal sodium reabsorption associated with the PKG-1 signaling pathway.\(^19\) These findings might be indicative of the functional importance of the described genetic variations in PRKG1. Therefore, it is plausible that genetically modified activity of PKG-1 in the carriers of GAT allele might also modulate cardiac performance. Along these lines, in our study population, the carriers of both GAT alleles had significantly increased LV radial systolic function compared with noncarriers or heterozygotes, although the exact mechanisms underlying the observed association remain to be elucidated.

We assessed LV systolic deformation (strain) using the tissue Doppler imaging technique, which allows measuring the velocity of shortening and lengthening of the myocardial tissue.\(^20\) From these velocity measurements, regional strain can be derived. One-dimensional strain quantifies the actual deformation of the myocardium in longitudinal and radial directions.\(^20\) Longitudinal movement of the LV results from contraction of longitudinally oriented subendocardial and subepicardial fibers, whereas radial LV wall thickening mainly originates from contraction of circumferential fibers located in the midwall.\(^21\) Thus, separate analysis of the various components of LV systolic function might be important for understanding the progression of changes of LV systolic function at different stages of heart disease.\(^22\) Gould et al\(^23\) assessed the relation between the directional components of LV contraction and ejection fraction in 122 subjects with or without heart disease by using cardiac angiography. The contribution of the longitudinal and radial components to total cardiac work was 14\% and 40\%, respectively.\(^23\)
As we demonstrated previously in the general population, chronically elevated blood pressure increases LV load and is accompanied by enhanced LV radial systolic performance. In the long run, hypertension leads to LV hypertrophy and increased LV oxygen requirements. On the contrary, in Langendorff-perfused mice hearts, the negative inotropism, which is associated with the NO–cGMP–PKG-1 signaling pathway, was accompanied by lower myocardial oxygen consumption. We observed higher LV systolic radial strain in GAT homozygotes. However, we do not know whether this variant is associated with a gain or loss of function or whether the observed change in LV function is a primary or compensatory response. Therefore, further studies must be undertaken to elucidate how the studied genetic variants of PRKG1 might influence the function of cardiomyocytes.

The present study must be interpreted within the context of its limitations and strengths. LV systolic and diastolic function phenotypes are quantitative traits, which arise through complex interaction between multiple genes and hemodynamic and environmental factors, and are prone to measurement error. In the present study, a experienced observer performed all echocardiograms with high reproducibility. There was also a high degree of internal consistency between the results of the population-based and family-based analyses. The between-family components of the QTDT were not statistically significant for the rs1904694 G allele and GAT haplotype, which makes it unlikely that our results are driven by population stratification. In view of the physiological consistency in the phenotype–genotype relations, it is unlikely that our findings just arose by chance. Adjustment for multiple comparisons is usually recommended to avoid rejecting null hypotheses too readily. The theoretical basis for advocating routine adjustment for multiple comparisons is that chance serves as the first-order explanation for observed phenomena. This hypothesis undermines one of the basic premises of epidemiological research, which holds that human biology follows regular laws that may be studied through observation of populations. Moreover, if phenotypes are correlated as in the present study, then multiple testing is not indicated because each new test does not provide a completely independent opportunity for a type I error. Under such circumstances, adjustment for multiple comparisons is inappropriate.

**Perspectives**

LV systolic radial function was associated with common polymorphisms in PRKG1. LV radial strain was significantly higher in GAT homozygotes than in heterozygotes and
noncarriers. Further studies, including target sequencing, will be required to clarify the functional role of this gene. If experimental studies and longitudinal follow-up of LV function confirm the causality of this association, interference with PKG-I function might be a target for pharmacological intervention.

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Disclosures

None.

References

23. Kuznetsova et al. LV Strain and PRKGI

Novelty and Significance

What Is New?

- This is the first study that addresses the association between cGMP-dependent protein kinase 1 (PRKGI) polymorphisms and left ventricular (LV) function in a general population.
- LV systolic radial function was associated with common polymorphisms in PRKGI.

What Is Relevant?

- LV radial strain was significantly higher in GAT homozygotes than in heterozygotes and noncarriers.
- We might speculate that the higher LV systolic radial strain in GAT homozygotes might make their hearts more vulnerable to continuously increased myocardial performance and lower the biological liability threshold for developing heart failure. However, this hypothesis can only be ascertained in a follow-up study.

Summary

LV systolic radial function was associated with common polymorphisms in PRKGI. If experimental studies and longitudinal follow-up of LV function confirm the causality of this association, interference with cGMP-dependent protein kinase 1 function might be a target for pharmacological intervention.
Left Ventricular Radial Function Associated With Genetic Variation in the cGMP-Dependent Protein Kinase

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