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.

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 participants.5-8 Transmission of the 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.

Study Participants

The Ethics Committee of the University of Leuven approved the FLEMENGO study. As described in detail in previous publications,9-11 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 reintivated 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.\(^5,6\) using a Vivid7 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 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.\(^7\) End-diastolic LV volumes 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 transmural 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 transmural 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 imaging images, using dedicated software as previously described.\(^8\) The SPEQUELE 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 measurements 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.

Genotypes
We extracted DNA from white blood cells. For genotyping of PRKG1 SNPs (rs1904694, rs7897633, and rs7905063), we used the 5′ nuclease allelic discrimination assays with allele-specific minor groove binding probes (TaqMan SNP Genotyping Assay-Applied Biosystem Inc, Foster City, CA) as described in detail elsewhere.\(^4\)

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\(^9,10\) 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\(^11\) 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>Clinical measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometrics</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>Questionnaire data, n (%)</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>Echocardiographic measurements</td>
<td></td>
<td></td>
</tr>
<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>4.80±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>Transmitral 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>7.1±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±11.8*</td>
</tr>
</tbody>
</table>

Genotypes and Haplotypes Frequencies

Table 2 lists the chromosome position, location type, and the allele frequencies of the selected tag SNPs of PRKG1 (rs1904694, rs7897633, and rs7905063). These 3 SNPs in PRKG1 span 59 kb from intron 1 to intron 2 and are located at chromosome 10q11.23. The genotype frequencies of the tag SNPs are shown in Table 3, and they complied with Hardy–Weinberg equilibrium (P≥0.28). The r² values reflecting linkage disequilibrium between these tag SNPs were 0.52 between rs1904694 and rs7897633, 0.73 between rs7897633 and rs7905063, and 0.77 between rs1904694 and rs7905063. Haplotype frequencies for ACC, GAT, AAT, AAC, and GCT were 41.7%, 34.4%, 10.1%, 8.1%, and 3.8%, respectively.

Population-Based Association Study

While accounting for family clusters and adjusting for important covariables, 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...
calcium current14,15 and phosphorylation of troponin I.16,17 The myocyte calcium responsiveness, such as reduction of L-type force of contraction by ≈(8-Br-cGMP and 8-pCPT-cGMP) reduced the cardiomyocyte negative inotropic effect. In this study, the cGMP analogues 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 (Δ 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 Doppler imaging technique, which allows measuring the velocity of shortening and lengthening of the myocardial

### Table 3. LV Radial Strain (%) by PRKG1 SNPs

<table>
<thead>
<tr>
<th>LV Radial Strain</th>
<th>Allele</th>
<th>Number of Coded Allele</th>
<th>00</th>
<th>01</th>
<th>11</th>
<th>PValue*</th>
<th>PValue†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1904694</td>
<td>G</td>
<td>Unadjusted, %</td>
<td>232 (38.0)</td>
<td>280 (45.8)</td>
<td>99 (16.2)</td>
<td>0.0010</td>
<td>0.0012</td>
</tr>
<tr>
<td>rs7897633</td>
<td>A</td>
<td>Unadjusted, %</td>
<td>142 (23.2)</td>
<td>297 (48.6)</td>
<td>172 (28.2)</td>
<td>0.0016</td>
<td>0.0006</td>
</tr>
<tr>
<td>rs7905063</td>
<td>T</td>
<td>Unadjusted, %</td>
<td>165 (27.1)</td>
<td>292 (47.7)</td>
<td>154 (25.2)</td>
<td>0.0053</td>
<td>0.0034</td>
</tr>
<tr>
<td>Haplotype</td>
<td>GAT</td>
<td>Unadjusted, %</td>
<td>265 (43.3)</td>
<td>273 (44.7)</td>
<td>73 (12.0)</td>
<td>0.0004</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Values are least square means±SE adjusted for family clusters, sex, age, body mass index, systolic blood pressure, and antihypertensive treatment. LV indicates left ventricle; and SNP, single-nucleotide polymorphism.

*P values are for the differences across the PRKG1 genotypes.
†P values are for the differences between homozygotes (11) and the rest of the PRKG1 genotypes (recessive model).
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 \textit{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, 1 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 \textit{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-1 function might be a target for pharmacological intervention.

**Acknowledgments**

We gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Hanne Tuyrens, and Daisy Thijs (Leuven, Belgium).

**Sources of Funding**

The European Union (grants IC15-CT98-0329-EPOGH, LSHM-CT-2006-037093-Genious, HyperCare, HEALTH-F4-2007-201500-HyperGenes, HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and European Research Council Advanced Grant-2011-294713-EPLOR) supported the Studies Coordinating Centre (Leuven, Belgium). The Studies Coordinating Centre also received grants from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium. The Studies Coordinating Centre also received grants from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13, and G. 0881.13).

**Disclosures**

None.

**References**


**Novelty and Significance**

**What Is New?**

- This is the first study that addresses the association between cGMP-dependent protein kinase 1 (PRKG1) polymorphisms and left ventricular (LV) function in a general population.
- LV systolic radial function was associated with common polymorphisms in PRKG1.

**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 PRKG1. 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

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

Hypertension. published online September 23, 2013;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2013/09/09/HYPERTENSIONAHA.113.01630

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/