

Hypertension

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Hypertension 2001;38;1278-1281

DOI: 10.1161/hy1101.096114

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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Angiotensin II Type 2 Receptors and Cardiac Hypertrophy in Women With Hypertrophic Cardiomyopathy

Jaap Deinum, Jeanette M.G. van Gool, Marcel J.M. Kofflard, Folkert J. ten Cate, A.H. Jan Danser

Abstract—The development of left ventricular hypertrophy in subjects with hypertrophic cardiomyopathy (HCM) is variable, suggesting a role for modifying factors such as angiotensin II. Angiotensin II mediates both trophic and antitrophic effects, via angiotensin II type 1 (AT₁-R) and angiotensin II type 2 (AT₂-R) receptors, respectively. Here we investigated the effect of the AT₂-R gene A/C³¹²³ polymorphism, located in the 3' untranslated region of exon 3, on left ventricular mass index (LVMI) in 103 genetically independent subjects with HCM (age, 12 to 81 years). LVMI and interventricular septum thickness were determined by 2D echocardiography. Extent of hypertrophy was quantified by a point score (Wigle score). Plasma prorenin, renin, and ACE were determined by immunoradiometric or fluorometric assays, and genotyping was performed by polymerase chain reaction. In men, no associations between AT₂-R genotype and any of the measured parameters were observed, whereas in women, LVMI decreased with the number of C alleles (211±19, 201±18, and 152±10 g/m² in women with the AA, AC, and CC genotype, respectively; *P*=0.015). Similar C allele-related decreases in women were observed for interventricular septum thickness (*P*=0.13), Wigle score (*P*=0.05), plasma renin (*P*=0.03), and plasma prorenin (*P*=0.26). Multiple regression analysis revealed that the AT₂-R C allele-related effect on LVMI ($\beta = -30.7 \pm 11.1$, *P*=0.010) occurred independently of plasma renin, the AT₁-R gene A/C¹¹⁶⁶ polymorphism, or the ACE gene I/D polymorphism. In conclusion, AT₂-Rs modulate cardiac hypertrophy in women with HCM, independently of the circulating renin-angiotensin system. These data support the contention that AT₂-Rs mediate antitrophic effects in humans. (*Hypertension*. 2001;38:1278-1281.)

Key Words: cardiomyopathy ■ hypertrophy ■ receptors, angiotensin II ■ renin

Hypertrophic cardiomyopathy (HCM) is characterized by idiopathic myocardial hypertrophy. It often occurs as an autosomal dominant disorder, but sporadic cases exist. Mutations in 8 different genes, all coding for sarcomeric proteins, have been identified in patients with HCM.¹ Patients vary considerably by phenotype, even if they have identical causative genotypes. This has led to the idea that trophic and mitotic factors modify the clinical manifestations of HCM.²

One of these factors is angiotensin (Ang) II. Ang II is generated by ACE from Ang I, which is formed by renin from angiotensinogen. Angiotensin production in the heart depends on kidney-derived renin and/or prorenin.^{3,4} Both are taken up from the circulation, either through diffusion into the cardiac interstitium or by binding to cardiac cells, and prorenin is activated to renin in cardiac cells.^{5,6} The extent of hypertrophy in subjects with HCM is associated with the ACE I/D polymorphism and the angiotensinogen M235T polymorphism,^{7,8} although the association may depend on the underlying disease gene mutation.⁹ Moreover, the Ang II type 1 receptor (AT₁-R) A/C¹¹⁶⁶ polymorphism also modulates the phenotypic expression of hypertrophy in subjects with HCM.¹⁰ AT₁-Rs mediate most, if not all, of the known effects

of Ang II, including vasoconstriction and growth stimulation. Taken together, these data support the concept of Ang II modifying cardiac hypertrophy in HCM.

In addition to its effects mediated via AT₁-Rs, Ang II also stimulates Ang II type 2 receptors (AT₂-Rs). Although AT₂-Rs are upregulated in the human heart under pathological conditions,^{11,12} their effects in man are currently unknown. Animal studies suggest that AT₂-R stimulation may oppose AT₁-R-mediated effects, ie, may result in vasodilation and growth inhibition.¹³⁻¹⁵ AT₂-R stimulation will occur in patients during treatment with AT₁-R antagonists, because the latter drugs increase the levels of Ang II.

In the present study, we set out to study the role of AT₂-Rs in man by investigating the association between a polymorphism in the 3' untranslated region of exon 3 (A/C³¹²³) of the X chromosome-located AT₂-R gene¹⁶ and the extent of cardiac hypertrophy in 103 HCM patients.

Methods

Patients

One hundred sixteen patients with HCM (age, 21 to 81 years) visiting the HCM Clinic at the Academic Hospital Dijkzigt between

Received January 20, 2001; first decision April 16, 2001; revision accepted June 13, 2001.

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TABLE 1. Characteristics of HCM Patients According to AT₂-R Genotype

Parameter	Men AT ₂ -R Genotype			Women AT ₂ -R Genotype			
	A (n=31)	C (n=32)	P (t test)	AA (n=8)	AC (n=15)	CC (n=17)	P (ANOVA)
Age, y	50±3	43±2	0.07	43±6	48±4	52±4	0.47
Body surface area, m ²	1.97±0.02	1.92±0.02	0.12	1.73±0.05	1.64±0.04	1.71±0.04	0.38
IVS, mm	21.7±0.8	21.8±0.9	0.93	25.5±1.5	23.8±1.6	21.0±1.2	0.13
LVMI, g/m ²	170±10	182±10	0.39	211±19	201±18	152±10	0.015
Wigle score (1–10)	6.4±0.4	6.5±0.4	0.88	8.0±0.5	7.3±0.5	5.6±0.7	0.05
Gradient, mm Hg	36.6±7.1	46.8±7.1	0.31	20.4±11.4	62.5±11.2	66.8±10.0	0.03
Prorenin, mU/L	222±41	191±21	0.49	258±83	183±41	152±16	0.26
Renin, mU/L	28.1±6.2	23.7±2.1	0.64	33.2±9.0	15.9±2.7	18.0±2.9	0.03
ACE, U/L	10.1±0.5	10.8±0.6	0.40	9.7±0.4	8.9±0.5	9.4±0.8	0.58

Values are mean±SEM. IVS indicates interventricular septum thickness; Gradient, peak left ventricular outflow tract gradient.

1994 and 1997 for a routine follow-up were included. HCM had been diagnosed on the basis of echocardiographic criteria showing a nondilated, hypertrophied left ventricle (any wall thickness >15 mm) in the absence of known causes of left ventricular hypertrophy.¹⁷ Patients using ACE inhibitors (n=7) were excluded from the study because of interference with the ACE measurement. Of the remaining 109 subjects, 41 had a sporadic form of HCM and 50 had at least one other affected first-degree family member. The family history of HCM was unknown in 18 patients. To avoid potential bias introduced by the presence of genetically dependent samples (relatives), we randomly selected 1 patient per family. One patient was excluded because he had Klinefelter's syndrome (XXY genotype). This resulted in a final cohort of 103 genetically independent patients, of whom 30 were receiving a β -adrenergic antagonist, 44 a calcium-channel blocker, and 8 a diuretic. The study was approved by the internal review board, and patients gave informed consent.

Echocardiographic Methods

Two-dimensional echocardiography was performed with commercially available equipment (Toshiba Sonolayer). Images were recorded on videotape for offline analysis by 2 physicians who were blinded to the genotyping results. Interventricular septal thickness and left ventricular mass (LVM) were determined as described before.¹⁰ LVM was indexed (LVMI) to body surface area.

Peak left ventricular outflow tract gradient at rest was estimated using the modified Bernoulli equation.¹⁰ Because echocardiographic measurement of LVMI may not truly reflect the extent of hypertrophy and the involvement (or lack thereof) of the distal (apical) half of the septum or lateral wall, the extent of hypertrophy was also assessed by a semiquantitative point score (range, 0 to 10) method developed by Wigle et al.¹⁸

Biochemical Measurements

Prorenin and renin were quantified in peripheral venous blood using an immunoradiometric assay kit (Nichols Institute).¹⁹ Renin and prorenin are expressed as mU/L, using the human kidney renin standard MRC 68/356 as a reference. ACE activity was measured with a commercial kit (ACE Color).²⁰

Genetic Analysis

Peripheral leukocytes were used to isolate genomic DNA in H₂O using the QIAamp Bloodkit (QIAGEN Inc). The ACE I/D polymorphism and the AT₁-R A/C¹¹⁶⁶ polymorphism were determined as described before.¹⁰ The AT₂-R A/C³¹²³ polymorphism, an *AluI* restriction fragment length polymorphism, was determined according to Katsuya et al.¹⁶

Statistical Analysis

Data are expressed as mean±SEM. Analysis was performed with the SPSS 9.0 statistical package. Hardy-Weinberg equilibrium was tested by χ^2 test. Univariate and multiple regression analyses were conducted to determine the percentage of explained variance in LVMI that is accounted for by the genotypes of the candidate modifier genes and other variables. In the multiple regression analysis, the renin-angiotensin system gene polymorphisms, age, peak left ventricular outflow tract gradient, and renin concentration were tested as independent variables. Prorenin and ACE were excluded from this analysis because of their high correlations with renin ($r=0.680$, $P<0.001$) and ACE genotype ($r=0.389$, $P=0.003$), respectively.

Results

Table 1 lists the characteristics of the HCM patients by AT₂-R genotype. Genotype frequencies were in agreement with Hardy-Weinberg equilibrium. The percentage of patients taking β -adrenergic antagonists, calcium channel blockers, or diuretics did not differ between the various groups (data not shown). In men, no genotype-related differences were observed with regard to any of the measured parameters. Similarly, in women no relationship between AT₂-R genotype and age, body surface area, or plasma ACE was observed. However, LVMI, Wigle score, and plasma renin in women decreased in parallel with the number of C alleles. Peak left ventricular outflow tract gradient was higher in women carrying the C allele. Univariate regression analysis showed that AT₂-R genotype accounted for 10.5%, 17.8%, 8.8%, and 12.9% of the variability of interventricular septal thickness ($r=0.32$; $P=0.044$), LVMI ($r=0.42$; $P=0.007$), plasma renin ($r=0.30$; $P=0.063$), and peak left ventricular outflow tract gradient ($r=0.36$, $P=0.023$), respectively.

Subdivision of men and women by both AT₁-R and AT₂-R genotypes (Table 2), to further investigate the previously described AT₁-R C allele-related effect on LVMI, revealed that the latter effect was restricted to male carriers of the AT₂-R A allele. In women, using 2-factor ANOVA, no interaction could be demonstrated between the AT₁-R C allele and the AT₂-R C allele with regard to LVMI.

Multiple regression analysis (Table 3) showed that age, AT₂-R genotype, and peak left ventricular outflow tract

TABLE 2. LVMI (g/m²) According to AT₁-R and AT₂-R Genotype

AT ₂ -R Genotype	AT ₁ -R Genotype	
	AA	AC+CC
Men		
A	143±5 (n=17)	202±17 (n=14)
C	184±20 (n=14)	180±11 (n=18)
Women		
AA	207±31 (n=5)	216±4 (n=3)
AC	196±24 (n=7)	205±27 (n=8)
CC	155±11 (n=13)	142±21 (n=4)

Values are mean±SEM.

In men, LVMI variation is explained for 6.2% ($P=0.04$) by the AT₁-R genotype. In women, LVMI variation is explained for 18.5% ($P=0.03$) by the AT₂-R genotype.

gradient, but not ACE genotype, AT₁-R genotype, or plasma renin, were significant predictors of LVMI in women.

Discussion

The present study shows that the AT₂-R genotype, in addition to the AT₁-R genotype,¹⁰ modulates the magnitude of LVMI in HCM patients. To our knowledge, these data are the first to demonstrate an AT₂-R-related effect on growth in the diseased human heart. Interestingly, the Ang II-mediated effects on LVMI appear to be the result of a balance between AT₁-Rs and AT₂-Rs, in a gender-specific manner, in that the AT₁-R C allele-related effect is observed in men carrying the AT₂-R A allele only, whereas the AT₂-R C allele-related effect is observed in women only, irrespective of their AT₁-R genotype. The concept of the net effect of Ang II being the result of the balance between AT₁-Rs and AT₂-Rs, AT₂-Rs opposing the growth-stimulatory effects of AT₁-Rs, originates from studies in whole animals^{21,22} and isolated cells.¹⁴

The polymorphic markers that we tested in the AT₁-R and AT₂-R genes, respectively, are located in untranslated regions. Consequently, their association with LVMI must be explained by a linkage disequilibrium with a functional variant of the 2 genes. Both receptors are expressed in the heart, with AT₁-Rs predominating under normal conditions.^{11,12} Initially, it was thought that AT₂-Rs are widely expressed in the fetal heart and disappear after birth, to return only under pathological conditions.²³ More recent studies in adult animals, however, have shown that this may not be true.^{24,25} In cardiomyopathic hamsters, AT₂-Rs exert an anti-AT₁-R action on the progression of interstitial fibrosis during

cardiac remodelling, by inhibiting both fibrillar collagen metabolism and growth of cardiac fibroblasts,²¹ whereas in the infarcted rat heart, AT₂-R blockade abolishes the beneficial effects of AT₁-R blockade.²² In the line of these animal data and the findings of the present study, it is logical to assume that AT₂-R stimulation in HCM protects against left ventricular hypertrophy, particularly in women with the CC phenotype.

The gender-specificity of the association of the AT₁-R and AT₂-R genotypes with LVMI is not readily explained. It may relate to the fact that the AT₂-R is located on the X-chromosome and thus is present twice in women only. The AT₂-R promoter region, unlike the angiotensinogen promoter region, does not contain estrogen-responsive elements, which suggests that if estrogens play a role in the development of left ventricular hypertrophy through AT₂-Rs, a third factor is involved, eg, an estrogen-dependent transcription factor. One may argue that the estrogen-induced higher angiotensinogen levels in women, via increased cardiac Ang II generation, have resulted in more intense AT₂-R stimulation. However, the lower plasma renin levels in women do not support this possibility.²⁶

The present study may be important from a pharmacotherapeutic point of view. If AT₁-R stimulation is indeed partly responsible for the increased LVM in subjects with HCM, the use of ACE inhibitors or AT₁-R antagonists in this disease might be reconsidered. Both are currently not widely used in HCM, although they are very effective in regressing and preventing ventricular hypertrophy in hypertension and after myocardial infarction. Prevention of hypertrophy in HCM is desirable in view of the increased risk of sudden death with higher LVM in subjects with HCM.²⁷ The antihypertrophic effect of AT₂-Rs, based on the current and previous data, raises the possibility that AT₁-R antagonists should be preferred above ACE inhibitors, because the former drugs, unlike ACE inhibitors, will result in AT₂-R stimulation. In support of this concept, Lim et al²⁸ recently demonstrated that AT₁-R blockade reverses myocardial fibrosis in a transgenic mouse model of human HCM.

In view of our present findings, one may also speculate on the role of AT₂-R genotypes in other hypertrophic conditions like hypertensive left ventricular hypertrophy and postinfarction remodelling. Schmieder et al²⁹ studied the A/G¹⁶⁷⁵ variant of the AT₂-R gene in 120 young males with normal or mildly elevated blood pressure and found LVMI to be higher in hypertensives with the A allele than in hypertensives with the G allele. It would be of interest to repeat this study in a larger population and preferably also in women.

Finally, the plasma levels of renin were partly determined by the AT₂-R genotype in women, and a similar trend was observed for the plasma levels of prorenin. Plasma renin however did not contribute independently to LVMI. These findings suggest that AT₂-Rs, like AT₁-Rs, affect the plasma levels of renin, but that at the same time cardiac Ang II generation (and thus cardiac AT₂-R stimulation) does not correlate directly with plasma renin levels. The latter might be explained on the basis of differences in the cardiac uptake of circulating renin, as well as differences in the uptake and local activation of circulating prorenin.^{5,6} The decrease of

TABLE 3. Multiple Regression Analysis of Factors With Potential Effect on LVMI in Women

Parameter	β	SEM	P
Age, y	-1.794	0.488	0.001
ACE genotype, No. of D alleles	-9.837	11.529	0.400
AT ₁ -R genotype, No. of C alleles	5.862	10.696	0.587
AT ₂ -R genotype, No. of C alleles	-30.691	11.148	0.010
Renin, mU/L	0.065	0.488	0.895
Gradient, mm Hg	0.445	0.180	0.019

LVMi with age, and its increase with peak left ventricular outflow tract gradient have been described before.^{10,30}

In summary, this paper suggests that the variability and/or extent of cardiac hypertrophy in HCM patients is partly determined by the balance between stimulation of AT₁-Rs and AT₂-Rs, with different effects in men and women. This may have therapeutic consequences for the prevention of hypertrophy in these patients, which may be important in view of the association of hypertrophy with sudden death in HCM.

Acknowledgment

This study was supported by a grant from The Netherlands Heart Foundation, No. NHS 97.186.

References

- Marian AJ, Roberts R. Molecular genetics of hypertrophic cardiomyopathy. *J Cardiac Electrophysiol.* 1998;9:88–99.
- Marian AJ. Pathogenesis of diverse clinical and pathological phenotypes in hypertrophic cardiomyopathy. *Lancet.* 2000;355:58–60.
- Danser AHJ, van Kats JP, Admiraal PJJ, Derckx FHM, Lamers JMJ, Verdouw PD, Saxena PR, Schalekamp MADH. Cardiac renin and angiotensins: uptake from plasma versus in situ synthesis. *Hypertension.* 1994;24:37–48.
- Danser AHJ, van Kesteren CAM, Bax WA, Tavenier M, Derckx FHM, Saxena PR, Schalekamp MADH. Prorenin, renin, angiotensinogen and ACE in normal and failing human hearts: evidence for renin-binding. *Circulation.* 1997;96:220–226.
- de Lannoy LM, Danser AHJ, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MADH. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart: local production of angiotensin I. *Hypertension.* 1997;29:1240–1251.
- van Kesteren CAM, Danser AHJ, Derckx FHM, Dekkers DHW, Lamers JMJ, Saxena PR, Schalekamp MADH. Mannose 6-phosphate receptor-mediated internalization and activation of prorenin by cardiac cells. *Hypertension.* 1997;30:1389–1396.
- Lechin M, Quinones MA, Omran A, Hill R, Yu QT, Rakowski H, Wigle D, Liew CC, Sole M, Roberts R, Marian AJ. Angiotensin I-converting enzyme genotypes and left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Circulation.* 1995;92:1808–1812.
- Ishano A, Okamoto H, Yoneya K, Watanabe M, Nakagawa I, Machida M, Onozuka H, Mikami T, Kawaguchi H, Hata A, Kondo K, Kitabatake A. Angiotensinogen gene polymorphism in Japanese patients with hypertrophic cardiomyopathy. *Am Heart J.* 1997;133:184–189.
- Tesson F, Dufour C, Moolman JC, Carrier L, Al-Mahdawi S, Chojnowska L, Dubourg O, Soubrier F, Brink P, Komajda M, Guicheney P, Schwartz K, Feingold J. The influence of the angiotensin I-converting enzyme genotype in familial hypertrophic cardiomyopathy varies with the disease gene mutation. *J Mol Cell Cardiol.* 1997;29:831–838.
- Osterop APRM, Kofflard MJM, Sandkuijl LA, ten Cate FJ, Krams R, Schalekamp MADH, Danser AHJ. AT₁ receptor A/C¹¹⁶⁶ polymorphism contributes to cardiac hypertrophy in subjects with hypertrophic cardiomyopathy. *Hypertension.* 1998;32:825–830.
- Wharton J, Morgan K, Rutherford RAD, Catravas JD, Chester A, Whitehead BF, De Leval MR, Jacoub MH, Pollack JM. Differential distribution of AT₂ receptors in the normal and failing human heart. *J Pharmacol Exp Ther.* 1998;284:323–336.
- Tsutumi Y, Matsubara H, Ohkubo N, Mori Y, Nozawa Y, Murasawa S, Kijima K, Maruyama K, Masaki H, Moriguchi Y, Shibasaki Y, Kamihata H, Inada M, Iwasaka T. Angiotensin II type 2 receptor is upregulated in human heart with interstitial fibrosis, and cardiac fibroblasts are the major cell type for its expression. *Circ Res.* 1998;83:1035–1046.
- Munzenmaier DH, Greene AS. Opposing actions of angiotensin II on microvascular growth and arterial blood pressure. *Hypertension.* 1996;27:760–765.
- van Kesteren CAM, van Heugten HAA, Lamers JMJ, Saxena PR, Schalekamp MADH, Danser AHJ. Angiotensin II-mediated growth and antigrowth effects in cultured neonatal rat cardiac myocytes and fibroblasts. *J Mol Cell Cardiol.* 1997;29:2147–2157.
- Carey RM, Wang Z-Q, Siragy HM. Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. *Hypertension.* 2000;35:155–163.
- Katsuya T, Horiuchi M, Minami S, Koike G, Santoro NF, Hsueh AJ, Dzau VJ. Genomic organization and polymorphism of human angiotensin II type 2 receptor: no evidence for its gene mutation in two families of human premature ovarian failure syndrome. *Mol Cell Endocrinol.* 1997;127:221–228.
- Maron BJ, Epstein SE. Hypertrophic cardiomyopathy: a discussion of nomenclature. *Am J Cardiol.* 1979;43:1242–1244.
- Wigle ED, Sasson Z, Henderson MA, Ruddy TD, Fulop J, Rakowski H, Williams WG. Hypertrophic cardiomyopathy: the importance of the site and the extent of hypertrophy: a review. *Prog Cardiovasc Dis.* 1985;28:1–83.
- Derckx FHM, de Bruin RJA, van Gool JMG, van den Hoek MJ, Beerenndonk CCM, Rosmalen F, Haima P, Schalekamp MADH. Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity. *Clin Chem.* 1996;42:1051–1063.
- Boomsma F, Schalekamp MADH. Evaluation of a test kit for the rapid and simple colorimetric measurement of angiotensin I-converting enzyme in serum. *J Clin Chem Clin Biochem.* 1983;21:845–849.
- Ohkubo N, Matsubara H, Nozawa Y, Mori Y, Murasawa S, Kijima K, Maruyama K, Masaki H, Tsutumi Y, Shibasaki Y, Iwasaka T, Inada M. Angiotensin type 2 receptors are reexpressed by cardiac fibroblasts from failing myopathic hamster hearts and inhibit cell growth and fibrillar collagen metabolism. *Circulation.* 1997;96:3954–3962.
- Liu Y-H, Yang X-P, Sharov V, Nass O, Sabbah HN, Peterson E, Carretero OA. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure: role of kinins and angiotensin II type 2 receptors. *J Clin Invest.* 1997;99:1926–1935.
- Matsubara H. Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ Res.* 1998;83:1182–1192.
- Wang Z-Q, Moore AF, Ozono R, Siragy HM, Carey RM. Immunolocalization of subtype 2 angiotensin II (AT₂) receptor protein in rat heart. *Hypertension.* 1998;32:78–83.
- Ozono R, Matsumoto T, Shingu T, Oshima T, Teranishi Y, Kambe M, Matsuura H, Kajiyama G, Wang Z-Q, Moore AF, Carey RM. Expression and localization of angiotensin subtype receptor proteins in the hypertensive rat heart. *Am J Physiol.* 2000;278:R781–R789.
- Danser AHJ, Derckx FHM, Schalekamp MADH, Hense H-W, Riegger GAJ, Schunkert H. Determinants of interindividual renin and prorenin variation: evidence for a sexual dimorphism of (pro)renin in humans. *J Hypertens.* 1998;16:853–862.
- Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. *N Engl J Med.* 2000;342:1778–1785.
- Lim D-S, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, Roberts R, Marian AJ. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. *Circulation.* 2001;103:789–791.
- Schmieder RE, Erdmann J, Delles C, Jacobi J, Fleck E, Hilgers K, Regitz-Zagrosek V. Effect of the angiotensin II type 2 receptor gene (+1675 G/A) on left ventricular structure in humans. *J Am Coll Cardiol.* 2001;37:175–182.
- Wigle ED, Rakowski H, Kimball BP, Williams WG. Hypertrophic cardiomyopathy: clinical spectrum and treatment. *Circulation.* 1995;92:1680–1692.