

In Search of Cardiovascular Candidate Genes Interactions Between Phenotypes and Genotypes

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Abstract—Most cardiovascular traits of interest can be defined as “complex traits,” with the first step in the identification of genetic factors affecting such traits being the detection of quantitative trait loci (QTLs). Animal models have proven particularly useful in this regard. However, only very few of the QTLs identified to date have led to the identification of candidate genes. We describe an example of our own work where the combination of anatomical and a biochemical intermediate phenotypes have led to the identification of the natriuretic peptide precursor A (*Nppa*) gene as a candidate gene for left ventricular hypertrophy (LVH). Combined with the power of comparative genetics, these strategies will continue to improve the chances of finding candidate genes for cardiovascular traits such as susceptibility to heart diseases, hypertension, and hypertension-induced end-organ damage. (*Hypertension*. 2002;39[part 2]:332-336.)

Key Words: genetics ■ hypertrophy, left ventricular ■ natriuretic peptides

“Complex traits” can be defined as traits in which (unlike Mendelian traits) there is not a simple one-to-one relationship between genotype and phenotype.¹ Although the identification of gene mutations linked to Mendelian traits is still a challenging task, it is a relatively achievable goal and depends mostly on the availability of informative pedigrees. Thus, as of September 2001, more than 900 syndromes have been entered in the “Online Mendelian Inheritance in Man” (OMIM) database as being linked to gene mutations (<http://www.ncbi.nlm.nih.gov/Omim/>). However, most cardiovascular traits of interest, including susceptibility to heart diseases, hypertension, and hypertension-induced end-organ damage, can be defined as complex traits. Genetic studies of multifactorial disorders in human populations remain challenging because of the multiplicity of genes underlying complex phenotypes, the modesty of the effect of each gene, and the heterogeneity that occurs within human populations.² Investigators have therefore relied on alternative strategies. In particular, efforts have been made to identify quantitative trait loci (QTLs) in crosses of inbred animals (mostly mice or rats), because alleles in the progeny of such crosses originate from only 2 possible sources (corresponding to the 2 parental strains), and a large numbers of chromosomal markers have become available for these models.

Recently, a reanalysis of linkage data obtained in 7 sets of intercrosses between 5 different inbred rat strains identified 67 QTLs for 39 blood pressure-related phenotypes.² These QTLs clustered in 15 independent genomic regions (on chromosomes 1 to 15), covering a distance of 567 centimor-

gan (cM), which corresponds to $\approx 30\%$ of the rat genome. These regions might not include all existing QTLs, because other ones have also been identified on chromosome 16 to 20, as well as on sex chromosomes.³ Positional cloning requires a QTL to be mapped to a region as small as 1 cM⁴ (equivalent to roughly 1 million bases in rodents), so there is still a long way to go between identification of a QTL and identification of a gene. In fact, a critical examination of the QTL literature reveals only 2 previous examples of a QTL leading to the cloning of a previously unknown gene, one in tomato⁵ and one in mice.⁵ There are also cases where identification of a QTL has led to the identification of a previously known gene that harbors sequence polymorphisms that might be responsible in part for variations in the quantitative trait.⁶⁻⁹ The difficulty of passing from QTL to gene identification has led some to develop alternative strategies, such as chemically-induced mutagenesis.¹⁰ At the very least, the examples listed above illustrate the fact that complementary strategies might be necessary for improving the chances of identifying genes linked to complex traits.

Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is a condition that has attracted considerable attention because it constitutes one of the most powerful independent risk factor for cardiovascular morbidity and mortality.^{11,12} In humans, cardiac mass is highly variable.¹³ Lifestyle (tobacco consumption, low levels of activity, diet, etc.) or underlying disease state (hypertension, diabetes, obesity, etc.) may contribute partly to the determination of cardiac mass,^{12,14-16} but such factors account for a surprisingly small portion of the variance.¹³ These

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data reveal the considerable effect of heritable factors and, more specifically, of “cardiac-mass-modifying” genes. In fact, it has been estimated (either in humans¹⁷ or in a sample of 23 rat inbred strains and crosses between them¹⁸) that the proportion of the blood pressure-independent variance of cardiac mass attributable to genetic factors was about 60%.

This is not to say that blood pressure does not contribute to increased cardiac mass. Although the correlation between clinical blood pressure and LV mass may be poor,^{19,20} it has been estimated that when blood pressure is measured by the ambulatory method, it may be responsible for up to 25% of the variance of LV mass in a given population.²¹ Likewise, several studies have reported on QTLs that were linked to both cardiac mass and blood pressure, using the progeny of crosses where one of the parental strains was hypertensive.^{22–25} Nonetheless, there is evidence that nonhemodynamic factors contribute importantly as well. For instance, LV mass has been shown to correlate weakly with aortic valve area in patients with aortic stenosis.¹³ From a genetic standpoint, it also has been possible to detect QTLs that are linked to cardiac mass independently of blood pressure in those crosses where one of the parental strains was hypertensive.^{24,26,27} However, because blood pressure is also a variable trait, it is consequently different in every member of the progeny of the genetic cross, which makes the interpretation of the results of such studies rather complex.

Linkage Studies for LVH in Normotensive Animals

One alternative has been to use crosses of normotensive strains that show quantitative differences in cardiac mass. For instance, crosses between the normotensive Wistar Kyoto (WKY) and F344 rats (where cardiac mass is higher in WKY) have been used to identify a QTL (on chromosome 3) that is significantly linked to cardiac mass.²⁸ However, that study did not lead to the identification of candidate genes linked to the trait of interest. Our laboratory has used crosses between WKY and Wistar Kyoto hyperactive (WKHA) rats, the latter one being a novel recombinant inbred strain derived from the progeny of F2 hybrid WKY/SHR rats.²⁹ We had observed that cardiac mass is about 10% higher in WKHA than in WKY rats,³⁰ whereas mean 24-hour systolic and diastolic blood pressure (as measured by 24-hour telemetry recordings in conscious animals³¹) was identical in both strains. LVH in WKHA was concentric in nature, because (1) the surface of the ventricular parenchyma was greater than in WKY, whereas there were no differences in the outer perimeter of the LV, and (2) the width/length ratio of isolated cardiomyocytes was higher in WKHA than in WKY.³¹ By testing the concentration of biochemical markers of LVH, we also found that the LV concentration of atrial natriuretic factor (ANF) mRNA and peptide were markedly lower in WKHA than in WKY.³⁰ Moreover, we found that low ventricular ANF concentration co-segregated with high LV mass in a segregating F2 cross-population, which demonstrated that both traits are genetically linked. Consequently, we used both phenotypes (LV mass and LV ANF) in a linkage study that used 345 F2 male rats originating from a cross between WKHA and WKY.⁹ We found significant evidence (LOD 12)

for a locus (QTL/ANFv) on chromosome 5 that was responsible for 44.3% of the total variance of log (LV ANF). There was also significant evidence (LOD 3.5) for a locus (QTL/LVM) on the same chromosome that was responsible for 18.6% of the total variance of LVM. The confidence intervals of QTL/ANFv and QTL/LVM were 12 and 28 cM, respectively, and overlapped a region that contains the locus of the natriuretic peptide precursor A (*Nppa*) gene³² (ie, the same gene that codes for the synthesis of ANF precursor).

Arguments in Favor of *Nppa* as a Candidate Gene for LVH

To consider a gene as a candidate for linkage to a genotype, several conditions should be met. (1) There should be molecular differences in the genes of the 2 parental strains; (2) the molecular differences should affect the level of expression of the genes or the function of the encoded proteins in a manner consistent with that seen in the 2 parental strains; and (3) the changes in expression level or protein function should make functional sense in relation to the phenotype of interest. To test the first condition, we sequenced for both strains 650 nucleotides of what has been reported as the minimal *Nppa* promoter³³ and found 2 single nucleotide polymorphisms. To test whether these polymorphisms altered the transcriptional activity of the promoter (as required to meet the second condition), we performed transfection experiments in cultured cardiomyocytes. We found that the transcriptional activity of the WKHA promoter was significantly lower than that of a promoter where the C base at position -93 had been substituted for a T base (as in WKY), which is in keeping with the fact that LV ANF is lower in WKHA than in WKY.

The last condition concerns the possible role of ANF in the context of LVH. Unlike our genetic WKHA and WKY models (where higher ventricular ANF correlates with low cardiac mass), it has been reported in many models of experimentally induced LVH that hypertrophy is generally accompanied by increases in the abundance of ANF mRNA.^{34–44} However, this should not be interpreted as meaning that ANF plays a causal role in LVH. In fact, recent evidence indicates that ANF (and/or its second messenger cGMP) may protect cardiac cells against hypertrophic stimuli. In vitro, the action of hypertrophic agents on cultured neonatal cardiac cells is inhibited by ANF and/or cGMP^{45–47} and is enhanced by an ANF antagonist.⁴⁸ In vivo, knockout inactivation of either *Nppa* or the natriuretic receptor A (NPRA) in mice increases ventricular mass disproportionately with the small changes in blood pressure observed in the same animals.^{49,50} Moreover, LVH develops in NPRA knockout mice independently of blood pressure,⁵¹ and the increased size of cardiac myocytes can be rescued by crossing the knockout mice with transgenic mice overexpressing a NPRA transgene in a heart-specific fashion.⁵² Finally, we have generated transgenic animals expressing constitutive guanylate cyclase in a heart-specific manner. Our preliminary data show that, in such mice, in vivo administration of either norepinephrine or isoproterenol induces significantly less hypertrophy than in wild-type animals (A. Zahabi, S. Picard, T.L. Reudelhuber, C.F. Deschepper, unpublished observa-

Complementary Phenotypes for QTL Studies

Type	Utility
Biochemical phenotypes	When the locus of the corresponding gene clusters with a QTL, it may provide clues for finding candidate genes linked to the complex trait.
Intermediate phenotypes	Decrease the genetic distance between genes and complex traits.
Alternative measurements	May increase the resolving power of linkage studies by increasing the statistical distance separating the mean values of the traits in the 2 parental strains.

QTL indicates quantitative trait locus.

tions). Altogether, these data are consistent with the hypothesis that increased ANF expression might constitute a compensatory response that may blunt (although not prevent) the effect of hypertrophic stimuli on cardiomyocytes. In this context, reduced ventricular expression of ANF (as in WKHA rats) might increase the sensitivity of the heart to hypertrophic agents and, thus, potentially lead to a facilitation for development of LVH. Conversely, a more robust level of ANF expression might be beneficial in the context of hypertrophic stimuli. This interpretation would be compatible with our observation in SHR and WKHT rats: although both strains are equally hypertensive, LV mass is higher in SHR than in WKHT, whereas ventricular ANF is lower in SHR than in WKHT.³⁰

Utility of Complementary Phenotypes in Linkage Studies

The above data provide an example in which the use of an additional phenotype has increased the information provided by a QTL alone. Indeed, if the QTL of a complex trait and that of an associated biochemical marker map to the same chromosomal region, it becomes likely that both traits are genetically linked. Moreover, if the biochemical phenotype is controlled by a gene whose locus maps to the same region, that gene becomes an attractive candidate, provided that its product has an effect on the complex trait of interest. Thus, when designing a linkage study, it is advantageous to collect as many biochemical phenotypes as possible in the organs associated with the complex trait of interest. This may be advantageous even after detecting a QTL. For instance, Karp et al have used microarrays to identify genes that were differentially expressed between animals from a cross with contrasting phenotypes and found that one such gene clustered with the QTL of interest.⁵³ On that basis, they repeated a linkage analysis, using single-nucleotide polymorphisms that were present in the genes of both parental strains. Combined with other functional assays, these studies enabled them to identify complement 5 as a susceptibility locus for experimental allergic asthma.⁵³

There are other types of secondary phenotypes that can be used in addition to the initial complex trait to increase the chances of detecting a QTL (Table 1). Indeed, complex traits are usually considered to be polygenic traits that result from the action of several "intermediate" traits that are themselves

controlled by a lower number of genes than the complex trait itself.⁵⁴ Some of such intermediate traits may even be Mendelian and, thus, may be controlled by just 1 gene. Each intermediate trait contributes in varying proportions (and sometimes, in association with others) to the final complex trait. If it is possible, on the basis of previous physiological studies, to incorporate in the study candidate intermediate phenotypes, it becomes possible to reduce the genetic distance between the trait and the causative genes and, thus, increase the chance of detecting a QTL. The second type of secondary phenotype is related to the procedure used to quantify the complex trait of interest, as exemplified in the study of Aitman et al.⁶ The aim of these investigators was to find QTLs linked to resistance to the metabolic effects of insulin and catecholamines in SHR. They proceeded to compare how 7 different experimental phenotypes representative of the action of insulin and catecholamines would separate SHR from the contrasting WKY strain. Among these 7 different phenotypes, they found that the mean values of some of the variables in parental strains were separated by as little as 0.8 SD, whereas the means of some other variables were separated by as much as 5.1 SD. Using the most discriminating phenotypes, they detected 2 QTLs on chromosomes 4 and 12. These findings later led them to the identification of Cd36 as an insulin-resistance gene.⁷ In our own studies, we recently found that, although the mean values of LV mass in WKHA and WKY were separated by only 1.3 SD, other variables made it possible to obtain much greater discrimination between the groups. For instance, when one measures the width/length ratio of isolated cardiomyocytes from both strains, the mean values of that variable were separated by as much as 6.3 SD.³¹ It is therefore possible that such measurements will provide more statistical power to detect QTLs associated with cardiac mass in future studies.

Conclusions

Whole-genome scans of segregating crosses between well-chosen inbred animal models have proven to be a powerful tool for the detection of QTL linked to complex traits. However, the transition from QTL detection to gene identification has proven difficult. In the future, it can be expected that by (1) incorporating complementary phenotypes in the design of studies and (2) harnessing the power provided by the availability of whole-organism's genomes, as well as comparative genomics, some of these difficulties might be overcome.

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References

1. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037–2048.

2. Stoll M, Kwitek-Black AE, Cowley AW Jr, Harris EL, Harrap SB, Krieger JE, Printz MP, Provoost AP, Sassard J, Jacob HJ. New target regions for human hypertension via comparative genomics. *Genome Res.* 2000;10:473–482.
3. Rapp JP. Genetic analysis of inherited hypertension in the rat. *Physiol Rev.* 2000;80:135–172.
4. Rapp JP, Deng AY. Detection and positional cloning of blood pressure quantitative trait loci: is it possible? *Hypertension.* 1995;25:1121–1128.
5. Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. *Science.* 2000; 289:85–88.
6. Aitman TJ, Gotada G, Evans AL, Imrie H, Heath KE, Trembling PM, Truman H, Wallace CA, Rahman A, Doré C, Flint J, Kren V, Zidek V, Kurtz TW, Pravenec M, Scott J. Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nat Genet.* 1998;16:197–201.
7. Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majali KM, Trembling PM, Mann CJ, Shoulders CC, Graf D, St. Lezin E, Kurtz TW, Kren V, Pravenec M, Ibrahim A, Abumrad NA, Stanton LW, Scott J. Identification of *Cd36 (Fat)* as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet.* 1999;21:76–83.
8. Rubattu S, Lee-Kirsch MA, DePaolis P, Giligerti R, Gigante B, Lombardi A, Volpe M, Lindpainter K. Altered structure, regulation, and function of the gene encoding the atrial natriuretic peptide in the stroke-prone spontaneously hypertensive rat. *Circ Res.* 1999;85:900–905.
9. Deschepper CF, Masciotra S, Zahabi A, Boutin-Ganache I, Picard S, Reudelhuber T. Functional alterations of the *Nppa* promoter are linked to cardiac ventricular hypertrophy in WKY/WKHA rat crosses. *Circ Res.* 2001;88:222–227.
10. Nadeau JH, Frankel WN. The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. *Nat Genet.* 2000;25:381–384.
11. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham heart study. *N Engl J Med.* 1990;322:1561–1566.
12. Devereux RB, de Simone G, Ganau A, Roman MJ. Left ventricular hypertrophy and geometric remodeling in hypertension: stimuli, functional consequences and prognostic implications. *J Hypertens.* 1994; 12(suppl 10):S117–S127.
13. Nunez DJR, Clifford CP, Al-Mahdawi S, Dutka D. Hypertensive cardiac hypertrophy: is genetic variance the missing link? *Br J Clin Pharmacol.* 1996;42:107–117.
14. Gardin JM, Wagenknecht LE, Anton-Culver H, Flack J, Gidding S, Kurosaki T, Wong ND, Manolio TA. Relationship of cardiovascular risk factors to echocardiographic left ventricular mass in healthy young black and white adult men and women. The CARDIA study. *Circulation.* 1995;92:380–387.
15. Amann K, Rychlik I, Miltenberger-Milteny G, Ritz E. Left ventricular hypertrophy in renal failure. *Kidney Int.* 1998;68:S78–S85.
16. Lind L, Andersson PE, Andren B, Lithell HO. Left ventricular hypertrophy in hypertension is associated with the insulin resistance metabolic syndrome. *J Hypertens.* 1995;13:433–438.
17. Verhaaren HA, Schieken RM, Mosteller M, Hewitt JK, Eaves LJ, Nance WE. Bivariate genetic analysis of left ventricular mass and weight in pubertal twins (the Medical College of Wisconsin twin study). *Am J Cardiol.* 1991;68:661–668.
18. Tanase H, Yamori Y, Hansen CT, Lovenberg W. Heart size in inbred strains of rats. Part 1. Genetic determination of the development of cardiovascular enlargement in rats. *Hypertension.* 1982;4:864–872.
19. Neutel JM, Smith DHG. Hypertension: where have we gone wrong, and how can we fix it? *Am J Hypertens.* 1998;11:150S–157S.
20. Cohn JN. Arteries, myocardium, blood pressure and cardiovascular risk: towards a revised definition of hypertension. *J Hypertens.* 1998;16: 2117–2124.
21. Devereux RB, Pickering TG, Harshfield GA, Kleinert HD, Denby L, Clark L, Pregibon D, Jason M, Kleiner B, Borer JS, Laragh JH. Left ventricular hypertrophy in patients with hypertension: importance of blood pressure response to regularly recurring stress. *Circulation.* 1983; 68:470–476.
22. Gu L, Dene H, Deng AY, Hoebee B, Bihoreau M-T, James M, Rapp JP. Genetic mapping of two blood pressure quantitative trait loci on rat chromosome 1. *J Clin Invest.* 1996;97:777–788.
23. Kato N, Hyne G, Bihoreau M-T, Ganguier D, Lathrop GM, Rapp JP. Complete genome searches for quantitative trait loci controlling blood pressure and related traits in four segregating populations derived from Dahl hypertensive rats. *Mamm Genome.* 1999;10:259–265.
24. Innes BA, McLaughlin MG, Kapuscinski MK, Jacob HJ, Harrap SB. Independent genetic susceptibility to cardiac hypertrophy in inherited hypertension. *Hypertension.* 1998;31:741–746.
25. Kren V, Pravenec M, Lu S, Krenova D, Wang J-M, Wang N, Merriouns T, Wong A, St. Lezin E, Lau D, Szpirer C, Szpirer J, Kurtz TW. Genetic isolation of a region of chromosome 8 that exerts major effects on blood pressure and cardiac mass in the spontaneously hypertensive rat. *J Clin Invest.* 1999;99:577–581.
26. Pravenec M, Ganguier D, Schott J-J, Buard J, Kren V, Bila V, Szpirer C, Szpirer J, Wang J-M, Huang H, St. Lezin E, Spence MA, Flodman P, Printz M, Lathrop GM, Vergnaud G, Kurtz TW. Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains. *J Clin Invest.* 1995;96: 1973–1978.
27. Hamet P, Kaiser MA, Sun Y, Pagé V, Vincent M, Kren V, Pravenec M, Kunes J, Tremblay J, Samani NJ. *HSP27* locus cosegregates with left ventricular mass independently of blood pressure. *Hypertension.* 1996; 28:1112–1117.
28. Sebkhii A, Zhao L, Lu L, Haley CS, Nunez DJR, Wilkins MR. Genetic determination of cardiac mass in normotensive rats. Results from an F344xWKY cross. *Hypertension.* 1999;33:949–953.
29. Deschepper CF, Prescott G, Hendley ED, Reudelhuber TL. Genetic characterization of novel strains of rats derived from crosses between Wistar-Kyoto and spontaneously hypertensive rats and comparisons with their parental strains. *Lab Anim Sci.* 1997;47:638–646.
30. Masciotra S, Picard S, Deschepper CF. Cosegregation analysis in genetic crosses suggests a protective role for atrial natriuretic factor against ventricular hypertrophy. *Circ Res.* 1999;84:1453–1458.
31. Deschepper CF, Picard S, Thibault G, Touyz R, Rouleau JL. Characterization of left ventricular myocardium, isolated cardiomyocytes and blood pressure in WKHA and WKY rats. *Am J Physiol.* 2002;82:H149–H155.
32. Bihoreau MT, Ganguier D, Kato N, Hyne G, Lindpainter K, Rapp JP, James MR, Lathrop GM. A linkage map of the rat genome derived from three F2 crosses. *Genome Res.* 1997;7:434–440.
33. Durocher D, Grépin C, Nemer M. Regulation of gene expression in the endocrine heart. *Recent Prog Horm Res.* 1998;53:7–23.
34. Calderone A, Takahashi N, Izzo NJ Jr, Thaik CM, Colucci WS. Pressure- and volume-induced left ventricular hypertrophies are associated with distinct myocyte phenotypes and different induction of peptide growth factor mRNAs. *Circulation.* 1995;92:2385–2390.
35. Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci USA.* 1988;85:339–343.
36. Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K, Iwao H. Angiotensin II induces cardiac phenotypic modulation and remodeling in vivo in rats. *Hypertension.* 1995;25:1252–1259.
37. Omura T, Kim S, Takeuchi K, Iwao H, Takeda T. Transforming growth factor β 1 and extracellular matrix gene expression in isoprenaline-induced cardiac hypertrophy: effects of inhibition of the renin-angiotensin system. *Cardiovasc Res.* 1994;28:1835–1842.
38. Lattion A-L, Michel JB, Arnaud E, Corvol P, Soubrier F. Myocardial recruitment during ANF mRNA increase with volume overload in the rat. *Am J Physiol.* 1986;251:H890–H896.
39. Farivar RS, Crawford DC, Chobanian AV, Brecher P. Effect of angiotensin II blockade on the fibroproliferative response to phenylephrine in the rat heart. *Hypertension.* 1995;25(suppl II):809–813.
40. Holder E, Mitmaker B, Alpert L, Chalifour LE. Morphometry and muscle gene expression in hypertrophied hearts from polyomavirus large T antigen transgenic mice. *Am J Physiol.* 1995;269:H86–H95.
41. Milano CA, Dolber PC, Rockman HA, Bond RA, Venable ME, Allen LF, Lefkowitz RJ. Myocardial expression of a constitutively active α_{1B} -adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc Natl Acad Sci USA.* 1994;91:10109–10113.
42. Wasaki H, Koya D, Schoen FJ, Jirousek MR, Ways DK, Hoit BD, Walsh RA, King GL. Targeted overexpression of protein kinase C β 2 isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci USA.* 1997; 94:9320–9325.
43. Hunter JJ, Tanaka N, Rockman HA, Ross J Jr, Chien KR. Ventricular expression of a MLC-2v-ras fusion gene induces cardiac hypertrophy and selective diastolic dysfunction in transgenic mice. *J Biol Chem.* 1995; 270:23173–23178.

44. D'Angelo DD, Sakata Y, Lorenz JN, Boivin GP, Walsh RA, Liggett SB, Dorn GWI. Transgenic *Gαq* overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci U S A*. 1997;94:8121–8126.
45. Calderone A, Thaik CM, Takahashi N, Chang DLF, Colucci WS. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. *J Clin Invest*. 1998;101:812–818.
46. Cao L, Gardner DG. Natriuretic peptides inhibit DNA synthesis in cardiac fibroblasts. *Hypertension*. 1995;25:227–234.
47. Silberbach M, Gorenc T, Hershberger RE, Stork PJS, Steyger PS, Roberts CT Jr. Extracellular signal-regulated protein kinase activation is required for the anti-hypertrophic effect of atrial natriuretic factor in neonatal rat ventricular myocytes. *J Biol Chem*. 1999;274:24858–24864.
48. Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. *Hypertension*. 2000;35:19–24.
49. John SWM, Krege JH, Oliver PM, Hagaman JR, Hodgins JB, Pang SC, Flynn TG, Smithies O. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science*. 1995;267:679–681.
50. Oliver PM, Fox JE, Kim R, Rockman HA, Kim H-S, Reddick RL, Pandey KN, Milgram KN, Smithies O, Maeda N. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci U S A*. 1997;94:14730–14735.
51. Knowles JW, Esposito JW, Mao L, Hagaman JR, Fox JE, Smithies O, Rockman HA, Maeda N. Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. *Proc Natl Acad Sci U S A*. 2001;107:975–984.
52. Kishimoto I, Rossi K, Garbers DL. A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. *Proc Natl Acad Sci U S A*. 2001;98:2703–2706.
53. Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Köhl J, Wahl L, Kuperman D, Germer S, Aud D, Peltz G, Wills-Karp M. Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. *Nat Immunol*. 2000;1:221–226.
54. Rapp JP. Dissecting the primary causes of genetic hypertension in rats. *Hypertension*. 1991;18(suppl III):I-18–I-28.