A Genetic Locus Accentuates the Effect of Volume Overload on Adverse Left Ventricular Remodeling in Male and Female Rats

Emmanuelle Souzeau, Bastien Llamas, Sonia Bélanger, Sylvie Picard, Christian F. Deschepper

Abstract—Although increased left ventricular (LV) mass is highly predictive of cardiovascular morbidity and mortality in humans, it has never been verified in an experimental model that naturally occurring alleles linked to increased LV mass under basal conditions also associate with worsened cardiovascular prognosis. Because we have shown previously that locus *Cm24* on chromosome 5 was responsible for differences in LV mass between WKY and WKHA rats, we used WKY.WKHA-(*D5Rat45-D5Rat245*) congenic rats (where locus *Cm24* has been transferred from WKHA into WKY rats) to test how naturally occurring gene variants present in *Cm24* would, in addition to their effects under basal conditions, affect LV mass remodeling and/or function in the context of overload. Volume overload was induced in WKY, WKHA, and WKY.WKHA congenic rats by surgical creation of an aorto-caval fistula. In females, the fistula had no effect on the hearts of WKY rats, yet it induced dilated eccentric hypertrophy and isolated diastolic dysfunction in WKHA and WKY.WKHA congenic rats, along with signs of congestive heart failure. In males, the surgical maneuver induced only mild or inconsistent responses in WKY rats but had much more pronounced effects in WKHA and WKY.WKHA congenic rats. Altogether, our data show that a genetic locus that induces, under basal conditions, either mild or no concentric LV remodeling in either male or female rats, respectively, associates with LV dilatation and dysfunction in both sexes when the hearts are additionally challenged. (***Hypertension*. 2006;47:128-133.)**

Key Words: hypertrophy ■ myocardium ■ myocytes ■ heart failure ■ genetics ■ gender ■ natriuretic peptides

Concentric left ventricular (LV) hypertrophy (LVH) is a frequent complication of hypertension. In adult humans, concentric LVH is highly predictive of cardiovascular morbidity and mortality,[1,2] the relationship being so strong that cardiovascular risk increases continuously and proportionally with LV mass (LVM).[3] Although demographic and anthropometric variables (including blood pressure) affect 25% to 50% of the variance of LVM, the remaining 50% to 75% of the variance is accounted for by other (presumably genetic) factors.[2] With the goal of finding genetic factors linked to the regulation of LVM, we have previously studied crosses of WKY and WKHA inbred rats, where WKHA rats display LVH in comparison to WKY rats despite identical and normal blood pressures.[4] These efforts have allowed us to detect on rat chromosome 5 a quantitative trait locus (QTL) that was linked to LVM in the male (but not the female) progeny under basal conditions.[5] This QTL is now identified by the symbol *Cm24* in the Rat Genome Database (available online at http://rgd.mcw.edu/). In a follow-up study, we generated congenic strains where a small segment of chromosome 5 from WKHA rats (containing QTL *Cm24*) was introgressed into the genetic background of the WKY strain.[6] In one of these strains [designated as WKY.WKHA–(*D5Rat45-D5Rat245*)], cardiomyocytes (CMs) isolated from adult male hearts were wider than CMs isolated from the parental WKY strain.[7] This indicated that the small amount of genetic material that was transferred from WKHA rats was sufficient to induce features of LV concentric remodeling in male WKY.WKHA-(*D5Rat45-D5Rat245*) rats versus their WKY counterparts.

Despite the strong correlation revealed by human epidemiologic studies, it has never been verified in an experimental model that naturally occurring alleles linked to increased LVM under basal conditions also associate with worsened cardiovascular prognosis. Our congenic rats, therefore, provided us with the opportunity to test how the naturally occurring gene variants present in *Cm24* would, in addition to their effects under basal conditions, affect LV remodeling and/or function in the context of overload. To this end, we performed surgery to create aorto-caval fistulae (ACF) on WKY, WKHA, and WKY.WKHA-(*D5Rat45-D5Rat245*) rats. ACF is a maneuver that induces volume overload and cardiac remodeling and has been shown to be accompanied by progressive cardiac decompensation in rats.[8,9]

Methods

Animals and Surgical Procedures

All of the procedures on animals were approved by the Institut de Recherches Cliniques de Montréal (IRCM) Institutional Animal Care Committee and conducted according to guidelines issued by the

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Canadian Council on Animal Care. The nomenclature of the strains is in compliance with the recommendations of the International Rat Genetic Nomenclature Committee. The WKHA/Cfd rats originated from a colony maintained at the IRCM, as registered with the Institute of Laboratory Animal Resources. WKY/Cfd rats also originated from a colony maintained at the IRCM and were derived from WKY/Cr parents obtained from Charles River (St. Constant, Québec, Canada). WKY.WKHA-(*D5Rat45-D5Rat245*) corresponded to the congenic strain described previously.[6] Adult animals were housed 2 to 3 per cage and given unlimited access to standard chow and water. All of the surgeries (either sham or ACF) were performed at 10 weeks of age, as described previously,[9,10] and animals euthanized 12 weeks after surgery, that is, at 22 weeks of age. Before euthanasia, the patency of the fistula was checked by visual inspection to exclude from the ACF group those animals where the fistula was no longer functional (an event that occurred in $<10\%$ of the operated animals).

Cardiac Morphology

The heart from each animal was removed with the ascending aorta attached. A PE90 catheter was inserted into the aorta above the level of insertion of the coronary arteries, and the heart was infused with a cold isotonic solution containing 100 mmol/L KCl and 50 mmol/L NaCl to arrest the heart in diastole. After diastolic arrest was obtained, the catheter was pushed further into the LV cavity and filled with the same solution to obtain an intracavital pressure of 15 mm Hg, as described previously.[4] The hearts were fixed in their distended form by immersion in formalin for 24 hours so that subsequent morphological comparisons were performed between hearts that had all been fixed under identical standardized intracavital pressure conditions. Sagittal sections ($\approx$2 mm) were cut at the midventricular level of all of the fixed hearts. Sections were examined on each side with a stereomicroscope, and images were captured as electronic files and analyzed using the Northern Eclipse version 6.0 software from Empix Imaging. For each heart section, the cross-sectional area (CSA) occupied by either LV wall tissue (including septum) or the LV cavity was calculated by averaging the values obtained for each side of the section. For determination of organ weight, lungs were weighed immediately after sacrifice, and biventricular cardiac weight was determined after the 24-hour fixation period. All of the CSA and organ weight values were normalized by dividing them by the value of tibia length. The latter was calculated by performing direct measurements on x-ray pictures of the hind legs of each animal.

Isolation of CMs and Videomicroscopy

CMs were isolated from the hearts of rats at 22 weeks of age, that is, 12 weeks after performing either sham surgery or creating ACF. The hearts were rapidly removed from anesthetized animals previously injected IP with 500 U heparin sulfate, and $[Ca^{2+}]$-tolerant CMs were isolated by the Langendorff method (cardiac retrograde aortic perfusion), as described previously.[4,11] CMs were separated from non-CMs by sedimentation at 1 unit of gravity in a 6% solution of bovine serum albumin, then fixed for 10 minutes in 0.08 mol/L phosphate buffer containing 1.5% glutaraldehyde at 4°C. Both solutions have been shown to preserve the volume of fixed cells as compared with unfixed ones.[12] Fixed CMs were allowed to settle in Petri dishes containing 0.15 mol/L phosphate buffer and examined with a Zeiss Axiovert microscope connected to a video camera that allowed capture of the images as electronic files. Using the Northern Eclipse version 6.0 software, $\approx$100 cells from each animal were analyzed for determination of cell length (defined as the longest length parallel to the longitudinal axis of the myocyte) and cell surface (calculated on the basis of the manual contour drawn around the myocyte). Cell width was calculated by dividing the value of surface by that of length.

Genetic Mapping

To additionally define the region containing genetic material of WKHA origin in our congenic strain, we performed additional analyses on genomic DNA with microsatellite markers, as described previously.[13] The mapped interval was then compared with the published sequence of rat chromosome 5 (build 3.1, available online at http://www.ncbi.nlm.nih.gov/mapview).

Hemodynamic Measurements

Cardiac functional variables were measured in sedated animals with a Fr-2 single sensor pressure catheter (Millar Instruments) and acquired with a PowerLab/8 nSP acquisition system (ADInstruments) as described previously.[9]

Statistical Analyses

The effects of strains and surgical procedures on all of the variables were assessed by 2-way ANOVA followed by Fisher's least significant difference (LSD) post-hoc tests. In each type of animal, hemodynamic variables were compared between sham-operated and ACF animals by Student *t* tests.

Results

By using additional microsatellite markers to map the precise boundaries of the WKHA locus introgressed within the congenic rat strain and comparing the results to the published sequence of rat chromosome 5 (build 3.1, available online at http://www.ncbi.nlm.nih.gov/mapview), we determined that the length of genetic material originating from WKHA is minimally of $\approx$5.5 Mb (comprising a total of 25 known genes) or maximally of $\approx$8.7 Mb (comprising a total of 39 known genes). The difference between the 2 intervals corresponds to regions of uncertainty where no polymorphic markers have been found yet, making it impossible to determine with certainty the exact boundaries of the locus from WKHA. A graphic representation of these results with the identity of the genes in the intervals is shown in Figure I, available online at http://www.hypertensionaha.org.

In 22-week-old females under basal conditions, signs of concentric LVH were present in WKHA but not in WKY.WKHA congenic rats. Accordingly, normalized LV CSA and normalized biventricular weight of WKHA were 12.5% and 15.9% higher than in their WKY counterparts, respectively (Figure 1). Likewise, CMs from WKHA were significantly wider than that from their WKY counterparts, as reflected by the decreased length/width ratio (Figure 3), but none of these values in female WKY.WKHA congenic rats were significantly different from their WKY counterparts. In female WKY rats, ACF had no effect on the morphology and/or weight of heart compartments. In contrast, ACF induced a significant increase of LV tissue CSA and normalized biventricular weight in female WKHA and WKY.WKHA rats (Figures 1 and 2). These changes were accompanied by a dilatation of the LV cavity and an increase in the relative length of CMs (as reflected by the increase in the length/width ratio; Figures 1, 2, and 3). Altogether, these findings indicate that ACF induced dilated eccentric LV hypertrophy in WKHA and WKY.WKHA female rats but not in female WKY. In addition, ACF increased lung weight and right ventricular (RV) CSA in female WKHA and WKY.WKHA congenic rats but not in their WKY counterparts. These changes represent signs of congestive heart failure and were accompanied by other qualitative signs detected at necropsy, that is, pleural effusions and a nutmeg appearance of the liver. Finally, ACF did not alter contractility indices in any strain of rats but induced an increase in τ (the

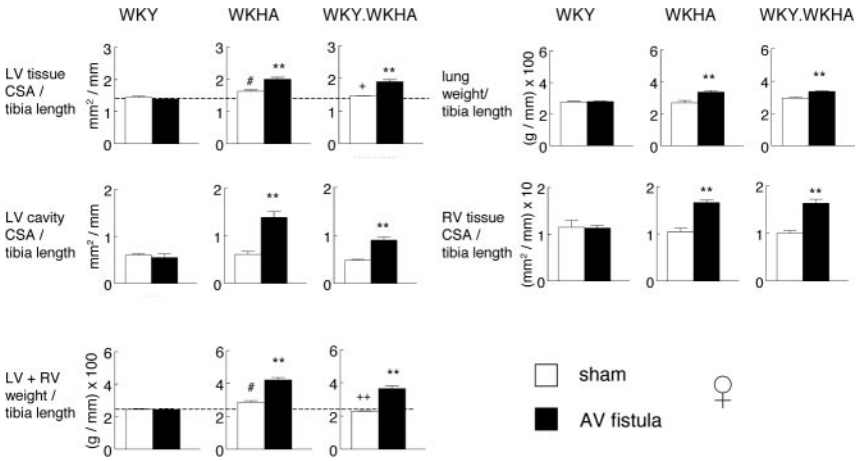

Figure 1. Effects of AV fistula on CSA variables, and on lung weight and biventricular (LV+RV) weight in WKY, WKHA, and congenic WKY.WKHA female rats. □, sham-operated animals; ■, animals with AV fistulae. Bar, mean±SEM (n=6 to 9 per group). □, sham surgery; ■, AV fistula. For each variable, the 2-way ANOVA revealed that the effects of surgery (factor A) and of strain (factor B) were significant ($P<0.001$) and that the interaction between surgery and strain was significant as well ($P<0.01$). Other symbols indicate differences found by Fisher's LSD post-hoc tests: $^{\#}P<0.05$ vs WKY; $^{*}P<0.05$ vs sham; $^{**}P<0.01$ vs sham; $^{+}P<0.05$ vs WKHA counterpart; $^{++}P<0.01$ vs WKHA counterpart.

time constant of LV pressure decay) in female WKHA and WKY.WKHA rats (Table), thus indicating that ACF induced isolated diastolic dysfunction in these animals.

At 22 weeks of age under basal conditions (sham operations), both WKHA and WKY.WKHA congenic rats each had some (but not identical) features of concentric LVH when compared with their WKY counterparts (Figures 3 and 4). In male WKHA rats, normalized LV tissue CSA, normalized biventricular weight, the CM surface area, and the CM relative width (as reflected by decreased length/width ratio) were all significantly higher (by ≈12% to 16%) than in hearts or CMs from their WKY counterparts. In male WKY.WKHA congenic rats, the CM surface area was significantly higher, and LV cavity surface was significantly lower than in their WKY counterparts. In contrast to females, ACF induced a mild but significant enlargement of the LV cavity (≈29%) and an increase in biventricular weight (≈20%) but no sign of congestive heart failure. In contrast, ACF had significantly greater effects on the morphology and weight of cardiac ventricular compartments of WKHA and WKY.WKHA, indicating that dilated ventricular hypertrophy developed much more prominently in these strains than in male WKY. Likewise, ACF increased the relative length of CMs (as reflected by the increase in the length/width ratio) only in WKHA and WKY.WKHA rats (Figure 3). Finally, signs of congestive heart failure (as evidenced by significant increase in lung weight and of RV CSA) were detected only in male WKHA and WKHA.WKY rats. As seen in females, ACF did not alter contractility indices in any strain of rats but induced an increase in τ (the time constant of LV pressure decay) in male WKHA and WKY.WKHA rats (Table 1), thus indicating that ACF induced isolated diastolic dysfunction in these animals.

Discussion

Hypertrophied left ventricles are remodeled most often along 1 of 2 different patterns, that is, concentric hypertrophy or dilated (also known as eccentric) remodeling.[14] In concentric hypertrophy, the thickness of LV walls is increased, whereas the size of the LV cavity is either unchanged or reduced. Dilated remodeling is characterized by an increase in the size of the LV cavity. In both types of remodeling processes, there is an increase in the size of CMs, although their shape is affected differently by either type of hypertrophic remodeling. Accordingly, the width of CMs increases disproportionately (as compared with their length) during concentric LVH, whereas dilated remodeling is characterized by CMs of which the length is increased disproportionately to their width.[15] Of

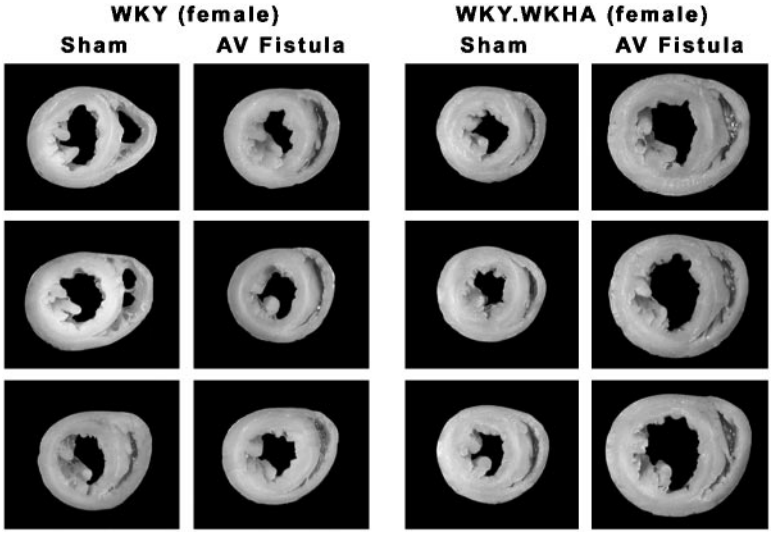

Figure 2. Representative images of sagittal sections of hearts from female WKY and WKY.WKHA rats arrested in diastole and fixed under pressurized conditions. All rats have been operated at 10 weeks of age either for sham surgery or for induction of an AV fistula. Whereas the AV fistula did not alter cardiac morphology in female WKY rats, it induced dilated eccentric hypertrophy in female WKY.WKHA rats along with an increase in RV CSA.

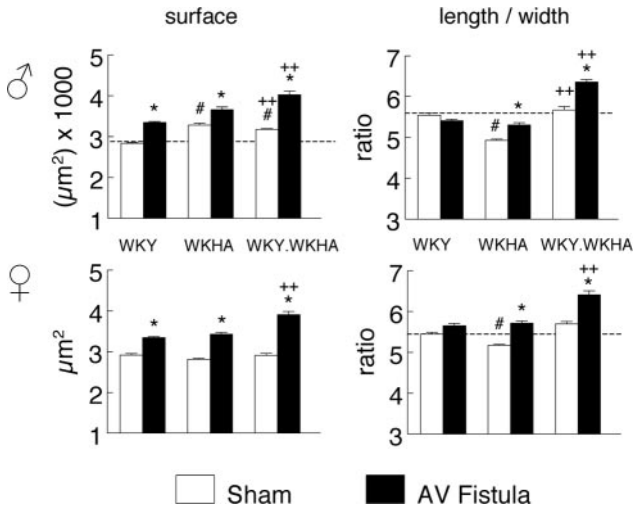

Figure 3. Effects of AV fistula on the morphology of CMs isolated from the hearts of male and female WKY, WKHA, and congenic WKY.WKHA rats. Each bar, mean±SEM (n=4 to 6 per group). □, sham surgery; ■, AV fistula. For each variable, the 2-way ANOVA revealed that the effects of surgery (factor A) and of strain (factor B) were significant ($P<0.001$) and that the interaction between surgery and strain was significant as well ($P<0.001$). Other symbols indicate differences found by Fisher's LSD post-hoc tests: $^{\#}P<0.05$ vs WKY; $^{*}P<0.05$ vs sham; $^{**}P<0.01$ vs sham; $^{++}P<0.01$ vs WKHA counterpart, either with sham surgery or AV fistula.

note, not all features of hypertrophy are always present at the same time in hypertrophied hearts.[16] Moreover, the distinction between both types of hypertrophic remodeling is sometimes blurred by the fact that concentric hypertrophy may progress toward dilated remodeling, particular as cardiac condition worsens.[17] Dilatation of the LV chamber appears to be a consistently deleterious feature, because it associates with increased wall stress and progression toward congestive heart failure[18–20] and, therefore, constitutes adverse ventricular remodeling.

We have shown previously that QTL *Cm24* on chromosome 5 was associated with LV concentric remodeling and/or increased width of CMs in 12-week-old male rats (but not in females) originating from crosses between the WKY and WKHA strains. To physically map that QTL, we have generated congenic rats where a small part of the genome of WKHA (centered on QTL *Cm24*) has been introgressed within the genetic background of WKY rats.[6] In the course of additional breeding and maintenance, that interval was additionally reduced. In the present study, we defined with greater accuracy the boundaries of this interval by additional mapping and determined that the length of chromosomic material of WKHA origin in our congenic strain is minimally of ≈5.5 Mb (comprising a total of 25 known genes), and maximally of ≈8.7 Mb (comprising a total of 39 known genes). The combined length of all autosomal chromosomes being of 2551 Mb, this interval, therefore, amounts to 0.21% to 0.34% of all autosomal genomic material. Compared with WKY rats, the transfer of the small locus from WKHA into WKY induced in male WKY.WKHA rats signs of concentric remodeling that were mild and less pronounced than in the parental WKHA rats, either in 12-week-old[6] or 22-week-old rats (in this study). In contrast, the same locus had no detectable effect on female WKY.WKHA rats under basal conditions.

ACF has been reported to induce cardiac remodeling and progressive congestive heart failure in male but not female Sprague-Dawley rats,[21] with that protection in females appearing to be dependent on circulating ovarian hormones.[22] The present data show that the genetic background plays an important role as well and, more precisely, that in the presence of allelic variant within locus *Cm24*, nonovariectomized female WKY.WKHA rats are not protected against ACF-induced adverse ventricular remodeling (both at the level of the heart and of CMs) and/or congestive heart failure. Although all types of male rats tested to date appear to be sensitive to the adverse effects of ACF, the present data also show that the genetic background (and, in particular, locus *Cm24*) modulates the extent to which male rats respond to ACF.

Hemodynamic Variables in Several Groups of Rats

Sex	Strain	Surgery	LVEDP (mm Hg)	LVESP (mm Hg)	+dP/dt (mm Hg/s)	−dP/dt (mm Hg/s)	τ (ms)
Male	WKY	Sham	1.32±0.30	125.3±4.1	9876±495	6808±588	18.6±0.74
Male	WKY	ACF	1.59±0.30	115.5±4.4	8304±596	6595±297	18.1±0.7
Male	WKHA	Sham	0.23±0.46	126±5.17	9127±286	5901±507	13.3±0.27
Male	WKHA	ACF	0.28±0.37	135±1.71	9597±465	6859±460	18.9±0.9*
Male	Cong.	Sham	0.52±0.27	139.9±4.8	10888±484	7570±476	7.5±0.6
Male	Cong.	Sham	0.33±0.32	134.3±5.9	10094±873	7490±227	13.2±1.8*
Female	WKY	Sham	1.66±0.62	129.7±0.8	10672±343	7503±375	11.1±0.5
Female	WKY	ACF	1.34±0.70	119.6±3.6	9547±295	6670±179	13.36±1.4
Female	WKHA	Sham	1.99±0.42	131±5.0	10484±492	6612±308	13.5±1.8
Female	WKHA	ACF	0.62±0.73	130±5.5	10070±611	7286±878	21.7±1.6*
Female	Cong.	Sham	0.22±0.25	120.3±2.4	9210±296	6589±296	7.5±0.9
Female	Cong.	Sham	0.33±0.18	128.6±2.9	9982±250	7195±480	12.1±1.9*

All values are mean±SE (n=5–8). LVDEP indicates LV end-diastolic pressure; LVESP, LV end-systolic pressure; Cong., congenic; +dP/dt and −dP/dt, maximum rate of positive and negative pressure change, respectively.

$^{*}P<0.05$ vs sham operated.

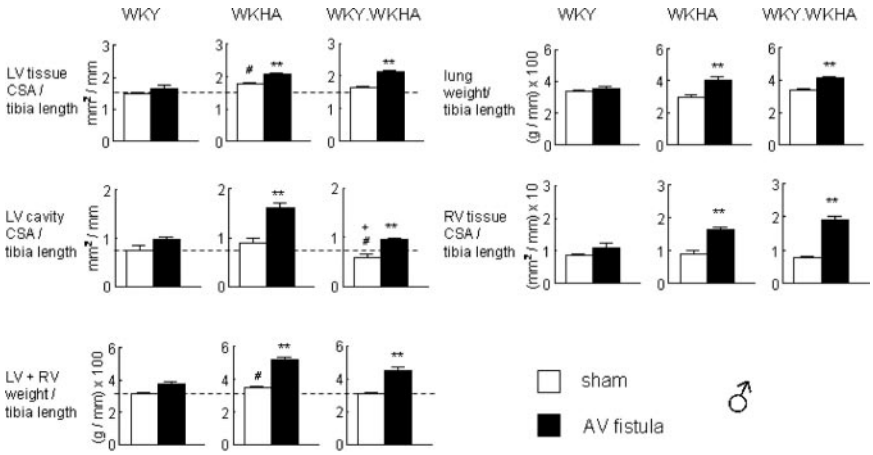

Figure 4. Effects of AV fistula on CSA variables and on lung weight and biventricular (LV+RV) weight in WKY, WKHA, and congenic WKY.WKHA male rats. □, sham-operated animals; ■, animals with AV fistulae. Each bar, mean±SEM (n=6 to 9 per group). □, sham surgery; ■, AV fistula. For each variable, the 2-way ANOVA revealed that the effects of surgery (factor A) and of strain (factor B) were significant ($P<0.001$) and that the interaction between surgery and strain was significant as well ($P<0.01$). Other symbols indicate differences found by Fisher's LSD post-hoc tests: $^{\#}P<0.05$ vs WKY; $^{*}P<0.05$ vs sham; $^{**}P<0.01$ vs sham; $^{+}P<0.05$ vs WKHA counterpart.

In both male and female WKHA and WKY.WKHA rats, ACF caused an increase in the LV cavity size (as measured under fixation at a standardized pressure of 15 mm Hg), which is indicative of increased ventricular compliance and constitutes a sign of LV diatolic dysfunction,[8,23] and an increase in the relative length of CMs, which has been shown to correlate closely with dilated remodeling and cardiac dysfunction in the course of deteriorating cardiac function.[15,24] Although we detected no sign of systolic dysfunction, ACF increased the τ constant of LV pressure decay in WKHA and WKY.WKHA rats, another sign of diastolic dysfunction. Of note, our data are compatible with those of others who have shown that chamber volume dilatation and diastolic function precede the appearance of LV dysfunction after ACF.[8] Dilatation in WKHA and WKY.WKHA is likely to be a manifestation of beginning congestive heart failure, as further evidenced by the fact that ACF induced an increase of lung weight and RV CSA in these 2 strains but not in WKY rats.

Contained within locus *Cm24* is the gene coding for the precursor of atrial natriuretic peptide (ANP), that is, natriuretic peptide precursor A (*Nppa*). We have shown previously that a mutation within the *Nppa* promoter associates with lower ventricular expression of *Nppa* in WKHA rats.[5] Additional convergent evidence obtained in transgenic and/or knockout animal models indicates that deficits in LV expression of *Nppa* are sufficient to explain changes in LVM and/or geometry.[25,26] Of note, it has been shown in knockout mice that inactivation of the ANP receptor altered the shape of CMs only in the presence of testosterone,[27] and we have shown that differences in the width of CMs of male WKHA and WKY rats were abolished when the animals had been castrated before puberty.[28] Thus, the hormonal status may explain, at least in part, why *Cm24* affects cardiac morphology in a sex-dependent fashion under basal conditions. Recent evidence indicates that the cardioprotective effects of ANP are not limited to LVM, but may extend to other cardiac insults.[29,30] These functional properties of ANP might explain why the effect of *Cm24* becomes evident in females only during overload conditions.

Perspectives

It has been shown previously that concentric LV geometry is the predominant and most dangerous adaptive pattern in arterial hypertension before the occurrence of any cardiovascular event.[31,32] The heritability of LVM is high, and twin studies have shown that genetic factors contribute ≈60% of the variance of LVM in either normotensive preadolescent subjects[33] or middle-aged adults.[34] Beyond the relatively rare cases of inherited cardiomyopathies (resulting from the mendelian inheritance of high-penetrance mutations of single genes),[14,35] little is known about the genetics of LVM.[34] The current data validate efforts aimed at elucidating the genetic determinants of common forms of LVH, because they show that a locus linked to concentric remodeling under basal conditions may also associate with progression toward dilated LV remodeling under overload conditions and, thus, with increased cardiovascular risk. From a genetic standpoint, our data in females also show that the effect of a specific locus may become detectable only under specific hemodynamic conditions.

Acknowledgments

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