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Topics: Population

Effects of genetic variation in adducin on left ventricular diastolic function as assessed by tissue Doppler imaging in a Flemish population

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Background **We investigated the possible association between left ventricular diastolic function and the *ADD1 Gly460Trp* and *ADD3 IVS11 +386A>G* polymorphisms alone and in combination.**

Methods **In a family-based population study (473 subjects; 50.5% women; mean age 50.5 years), we measured early (Ea) and late (Aa) diastolic peak velocities of the mitral annulus by tissue Doppler imaging. In multivariate-adjusted analyses, we investigated phenotype–genotype associations, while accounting for confounders and family structure.**

Results **Lateral Ea/Aa ratio was higher in *ADD1 Trp* allele carriers than in *GlyGly* homozygotes (1.51 vs. 1.40; $P = 0.005$) and was lower in *ADD3 A* allele carriers than in *GG* homozygotes (1.42 vs. 1.55; $P = 0.005$). The effects of *ADD1* on the lateral Ea and Ea/Aa weakened with older age ($P < 0.05$). The best fitting model for lateral Ea and Ea/Aa included *ADD1*, *ADD3*, and the three-way interaction term of both genes with age. Below the age of 50 years, the lateral Ea/Aa ratio was higher in *ADD1 Trp* allele carriers than in *GlyGly* homozygotes (1.91 vs. 1.73; $P = 0.006$), particularly in the presence of *ADD3 GG* homozygosity (2.46 vs. 1.80; $P = 0.0008$). In older subjects, these phenotype–genotype associations were not significant ($P > 0.20$). Transmission of the *ADD1 Trp* allele to offspring was associated with higher lateral Ea (+0.91; $P = 0.026$) and Ea/Aa ratio (+0.23; $P = 0.0008$).**

Conclusion **Our population-based study demonstrated that left ventricular diastolic relaxation is modulated by genetic variation in *ADD1* and *ADD3*. This association was more prominent in younger subjects in whom longstanding environmental factors and ageing are less likely to mask genetic effects.** *J Hypertens* **26:1229–1236**

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Keywords: adducing, echocardiography, general population, mitral annular tissue velocity, tissue Doppler imaging

Abbreviations: A, the transmitral peak late diastolic velocity; Aa, the peak late diastolic mitral annular velocity; ADD1, α–adducin gene; ADD3, γ–adducin gene; E, the transmitral peak early diastolic velocity; E/A ratio, E velocity/A velocity ratio; Ea, the peak early diastolic mitral annular velocity; Ea/Aa ratio, Ea velocity/a velocity ratio; FLEMENGHO, Flemish Study on Environment, Genes and Health Outcomes; IVRT, isovolumetric relaxation time; LV, left ventricle; NCX, Na^+Ca^{2+}–exchanger; QTDT, quantitative transmission disequilibrium test; SD, standard deviation; SE, standard error; SNP, single nucleotide polymorphism; TDI, Tissue Doppler Imaging

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Introduction

Adducin is a heterodimeric cytoskeleton protein consisting of an α-subunit and β-subunit or and α-subunit and γ-subunit that, to a large extent, are similar in amino acid sequence and domain organization [1]. Mutation of the α-adducin gene (*ADD1*) is associated with increased Na^+,K^+-ATPase activity [2,3] and increased renal tubular sodium reabsorption [4]. *ADD1* and the γ-adducin gene (*ADD3*) are not only expressed in the kidney but also in the heart [5]. Variation in the Na^+,K^+-ATPase activity and the intracellular Na^+ concentration might influence the sodium-dependent transmembranous Ca^{2+} transport in cardiomyocytes [6] and, therefore, the rate of myocardial relaxation, which partially depends on the removal of intracellular Ca^{2+} through the Na^+,Ca^{2+}-exchanger (NCX) [6]. Moreover, carriers of mutated *ADD1* have an increased risk of hypertension [7] and heart failure [8]. Left ventricular diastolic dysfunction is a forerunner of overt heart failure. We therefore investigated the possible association between left ventricular diastolic function, as assessed by transmitral Doppler flow velocities and mitral annular tissue velocities, and the *ADD1 Gly460Trp* and *ADD3 IVS11 +386A>G* polymorphisms alone and in combination. As ageing modifies penetrance of the rodent and human phenotypes associated with mutation of *ADD1* [4] and also leads to impairment of left ventricular diastolic

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function, we paid particular attention to the possible interaction of age with the genotypes under study.

Methods

Study participants

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes [7]. From August 1985 to December 2005, we randomly recruited a family-based population sample from a geographically defined area in northern Belgium [7,8]. We invited 672 participants for a follow-up examination at our field center, including echocardiography. After excluding 20 patients who were bedridden or institutionalized, we obtained informed written consent from 535 subjects (participation rate 82.0%). We discarded eight subjects from the analysis because their left ventricular diastolic function could not be assessed from their echocardiogram. We excluded a further 45 subjects because of left ventricular remodeling owing to myocardial infarction or coronary revascularization ($n = 13$), or because of valvular heart disease ($n = 27$), atrial fibrillation ($n = 3$), or presence of an artificial pacemaker ($n = 2$). The PCR reaction did not yield a reliable genotype in nine subjects. Thus, a total of 473 participants were statistically analyzed.

Echocardiography

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 h before echocardiography. The blood pressure recorded during echocardiography was the average of two readings obtained with a validated [9] OMRON 705IT device (Omron Corp., Tokyo, Japan) at the end of the examination.

Data acquisition

One experienced physician (Tatiana Kuznetsova) did the ultrasound examination using a Vivid7 Pro (GE Vingmed, Horten, Norway) interfaced with a 2.5–3.5-MHz phased-array probe according to the recommendations of the American Society of Echocardiography [10]. With the subjects in partial left decubitus position and breathing normally, she obtained images, together with a simultaneous ECG signal, from the parasternal long and short axes and the apical four-chamber and two-chamber long-axis views. All recordings included at least five cardiac cycles and were digitally stored for off-line analysis. M-mode echocardiograms of the left ventricle were recorded from the parasternal long-axis view under control of the two-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae.

To record mitral flow velocities from the apical window and the isovolumetric relaxation time, the observer positioned the Doppler sample volume at the mitral valve tips and between the left ventricular outflow and mitral inflow, respectively. Using tissue Doppler imaging (TDI), she recorded low-velocity, high-intensity myocardial signals at a high frame rate (>190 frames per second) while adjusting the imaging angle to ensure a parallel alignment of the ultrasound beam with the myocardial segment of interest. From the apical window, the sonographer placed a 5-mm Doppler sample at the septal, lateral, inferior site, and posterior site of the mitral annulus. The Nyquist limit was adjusted to 15–20 cm/s with minimal gain and low wall filter settings.

Off-line analysis

Two sonographers analyzed recorded images, averaging three heart cycles for statistical analysis, using a workstation running the EchoPac, version 4.0.4 (GE Vingmed) software package. The left ventricular internal diameter and interventricular septal and posterior wall thickness were measured at the end of diastole by a two-dimensionally guided M-mode tracing, as described in the American Society of Echocardiography guideline [10]. End-diastolic left ventricular dimensions were used to calculate the left ventricular mass by an anatomically validated formula. Left ventricular end-systolic and end-diastolic volumes were calculated by Teicholtz's method. We used the apical four-chamber view to measure the left ventricle length.

From the transmitral flow signal, the observer measured the peak early diastolic velocity (E), the peak late diastolic velocity (A), and the E/A ratio. Isovolumetric relaxation time was the time from the closure of the aortic valve to the onset of the mitral inflow. From the TDI recordings, she determined the peak early (Ea) and the peak late (Aa) diastolic mitral annular velocities and the Ea/Aa ratio at four acquisition sites (septal, lateral, inferior, and posterior).

To determine reproducibility, two experienced echocardiographists (Tatiana Kuznetsova and Lieven Herbots) read the recordings of 18 subjects. For each pair of single measurements, we first determined the relative difference between the observations of the two readers as $(x_1 - x_2)/(x_1 + x_2)/2 \times 100$. The interobserver reproducibility coefficient of a measurement was the 2 SD interval about the mean of the relative differences across 18 pairwise readings. Reproducibility across the four sampling sites ranged from 4.48 to 5.34% for Ea velocities and from 3.96 to 4.52% for Aa velocities (Supplemental Table S1).

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 a systolic blood pressure of at least 140 mmHg or diastolic blood pressure of 90 mmHg (average of five consecutive readings at the examination center) or the use of antihypertensive drugs. Body mass index was the ratio of weight (in kilograms) and the square of height (in meters).

Genotypes

We extracted DNA from white blood cells according to standard methods [7]. For genotyping, we used a 5′ nuclease detection assay implemented on an ABI Prism 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA). Applied Biosystems' Custom Assay-by-Design Service prepared specific TaqMan primers and probes for both *ADD1 Gly460Trp* (rs4961 dbSNP) and *ADD3 IVS11 +386A>G* (rs3731566 dbSfNP) polymorphisms. The specific sequences for rs4961 are: forward primer 5′-GAGAAGACAAGATGGCTGAACT CT-3′, reverse primer 5′-GTCTTCGACTTGGGACTG CTT-3′, and probes 5′-CATTCTGCCCTTCCTC-3′ and 5′-ATTCTGCCATTCCTC-3′. The sequences for rs3731566 genotyping are: forward primer 5′-AGGTGG-GAATTGAAGAGACTCTCA-3′, reverse primer 5′-CAA CTATGCAGATGACCTTTGCTTT-3′, and probes 5′-TCTGGAAATGTCAAATAGTAA-3′ and 5′-CTGGAA ATGTCAAGTAGTAA-3′.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, North Carolina, USA). We compared means and proportions by large sample z-test and χ^2-test, respectively. We normalized the distribution of the lateral Ea/Aa ratio by a square root transformation. We performed stepwise multiple regression to assess the independent correlations of transmitral and TDI mitral annular velocities with sex, age, height, weight, body mass index, heart rate, systolic and diastolic blood pressures during the echocardiographic examination, left ventricular length, left ventricular mass index, and ejection fraction. Variables with P values of 0.10 were used in the model.

We performed both population-based and family-based analyses. In the former approach, we used a mix model to test the association of dependent variables (transmitral and TDI mitral annular velocities and their ratios) with the genotypes of interest. This technique allows accounting for covariates and nonindependence of observations within families. For analysis of the *ADD1* single nucleotide polymorphism (SNP), we combined the less-frequent homozygous group (*TrpTrp*) with the heterozygous subjects (*GlyTrp*). As in our previous studies [8], we analyzed phenotype–genotype associations by comparing *ADD1 GlyGly* homozygotes with carriers of the mutated *Trp* allele and testing the differences across the three *ADD3* genotypes. Our study had 80% power to detect an effect size for the Ea/Aa ratio of 0.17 at an α-level of 0.05. For the pairwise comparison of *ADD3* genotypes, we applied Bonferroni's correction of the significance levels. We used likelihood ratio tests to evaluate the goodness of fit of nested models. In the family-based analyses, we used the orthogonal model proposed by Abecasis *et al.* [11] to evaluate the within-family and between-family components of phenotypic variability. We implemented the quantitative transmission disequilibrium test using a mixed model with similar adjustments as in population-based analyses. We tested the interactions between genotypes and between genotypes and age by introducing the appropriate interaction terms in the model.

Results

Characteristics of participants

Of the 473 participants included in the study, 239 (50.5%) were women and 176 (37.2%) were hypertensive patients of whom 95 (20.1%) were on antihypertensive drug treatment. Table 1 shows the clinical and echocardiographic characteristics of the study participants by sex. Women had lower systolic and diastolic blood pressures, higher heart rates, and less frequently reported intake of alcohol (Table 1) compared with men. The 24-h urinary sodium excretion averaged 144.9 mmol per day in women and 195.8 mmol per day in men.

Transmitral and tissue Doppler imaging velocities

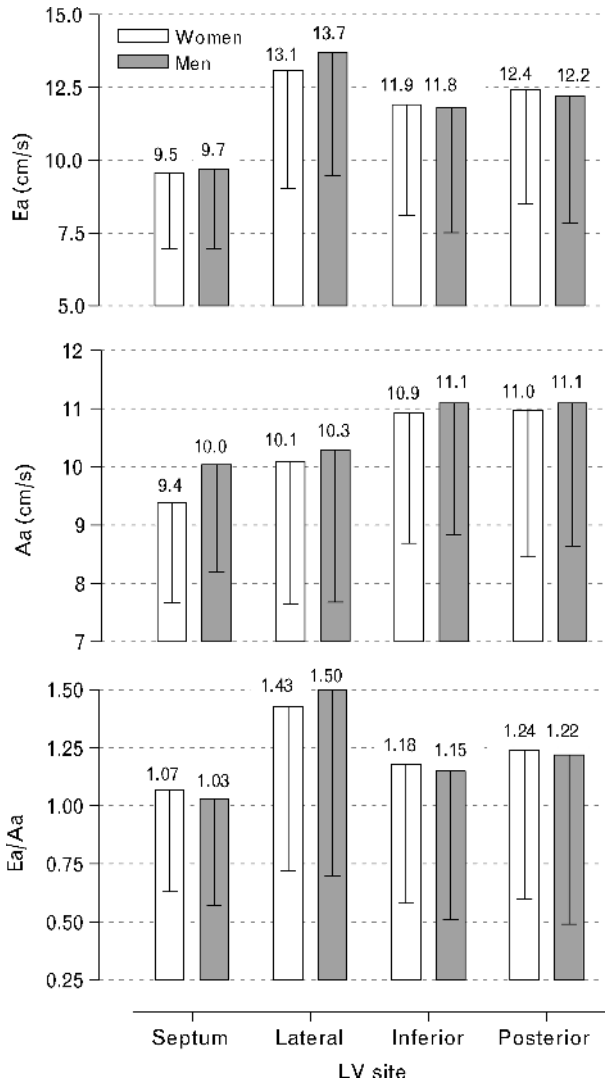
Transmitral peak flow velocities were greater in women, whereas the opposite was true for the echocardiographic measurements reflecting the left ventricular size (Table 1). In the whole study population, lateral Ea, Aa, and Ea/Aa ratio averaged 13.3 ± 4.1, 10.2 ± 2.6 cm/s, and 1.44 ± 0.75,

Table 1 Characteristics of participants

Characteristic	Women ($n = 239$)	Men ($n = 234$)
Clinical measurements		
Anthropometrics		
Age (years)	50.9 ± 13.8	50.1 ± 15.1
Height (cm)	162.9 ± 6.7	$175.2 \pm 7.0^{\dagger}$
Weight (kg)	69.1 ± 13.1	$81.0 \pm 10.8^{\dagger}$
Body mass index (kg/m^2)	26.0 ± 4.5	26.4 ± 3.4
Systolic pressure (mmHg)	129.6 ± 20.0	$133.0 \pm 14.8^{*}$
Diastolic pressure (mmHg)	76.2 ± 9.1	$77.8 \pm 9.0^{*}$
Heart rate (beats/min)	62.7 ± 9.0	$58.5 \pm 8.9^{\dagger}$
Questionnaire data		
Current smoking [*n* (%)]	54 (22.6)	56 (23.9)
Drinking alcohol [*n* (%)]	66 (27.6)	142 (60.7)†
Hypertensive patients [*n* (%)]	85 (35.6)	91 (38.9)
Treated for hypertension [*n* (%)]	52 (21.8)	43 (18.4)
Echocardiographic measurements		
Conventional echocardiography		
Left atrial diameter (cm)	3.73 ± 0.46	$4.12 \pm 0.52^{\dagger}$
LV internal diameter (cm)	4.80 ± 0.39	$5.25 \pm 0.43^{\dagger}$
Interventricular septum (cm)	0.92 ± 0.15	$1.05 \pm 0.18^{\dagger}$
Posterior wall (cm)	0.82 ± 0.14	$0.91 \pm 0.14^{\dagger}$
LV mass index (g/m^2)	82.4 ± 16.5	$98.6 \pm 19.7^{*}$
Ejection fraction (%)	69.6 ± 7.3	68.3 ± 7.2
LV length (cm)	7.72 ± 0.56	$8.65 \pm 0.62^{\dagger}$
Doppler mitral flow		
E peak (m/s)	0.81 ± 0.16	$0.73 \pm 0.15^{\dagger}$
A peak (m/s)	0.69 ± 0.16	$0.62 \pm 0.17^{\dagger}$
E/*A* ratio	1.25 ± 0.44	1.28 ± 0.50
IVRT (ms)	99.8 ± 15.8	102.7 ± 15.8

Values are mean (±SD) or number of subjects (%). LV and IVRT indicate left ventricle and isovolumetric relaxation times, respectively. * Significance of the sex difference: $P \leq 0.05$. † Significance of the sex difference: $P \leq 0.001$.

Fig. 1



Peak early (Ea) and late (Aa) diastolic mitral annular velocities and Ea/Aa ratio by acquisition site and sex

Ea, Aa, and Ea/Aa ratio were similar in 239 women and 234 men, with the exception of septal Aa, which was higher in men ($P = 0.0002$). Ea, Aa, and Ea/Aa ratio were significantly different ($P < 0.001$) across all acquisition sites with maximal values of Ea and Ea/Aa at the lateral site.

respectively. Ea and Ea/Aa were higher ($P < 0.0001$) at the lateral site than at the other acquisition sites (Fig. 1).

The transmitral *E/A* ratio and the lateral mitral annular Ea/Aa ratio both significantly and independently decreased with age, body mass index, heart rate, and diastolic blood pressure (Table 2). The transmitral *E/A* ratio, but not the lateral mitral annular Ea/Aa ratio, also increased with the ejection fraction. The total explained variance was 69.0% for the transmitral *E/A* ratio and 69.4% for the lateral mitral annular Ea/Aa ratio. Age accounted for most of the explained variance, 53.9 and 58.9%, respectively. The correlates of the peak transmitral and lateral mitral annular velocities are given in Table 2.

Population-based association study

The frequencies of the *ADD1* genotypes were 58.6% for *GlyGly*, 36.4% for *GlyTrp*, and 5.0% for *TrpTrp*; the frequencies of the *ADD3* genotypes were 29.2% for *AA*, 46.5% for *AG*, and 24.3% for *GG*. As homozygous carriers of the *ADD1 Trp* represented only 5.0% of the study participants, we contrasted *ADD1 Trp* allele carriers with *GlyGly* homozygotes.

Although accounting for family clusters, the lateral mitral annular Ea/Aa ratio was significantly higher in *ADD1 Trp* allele carriers than in *GlyGly* homozygotes before and after adjustment (Table 3) for age, body mass index, heart rate, and diastolic blood pressure measured immediately after the echocardiographic examination (1.51 vs. 1.40; $P = 0.005$). Moreover, the adjusted lateral Ea/Aa ratio was significantly lower in *ADD3 A* allele carriers than in *GG* homozygotes (1.42 vs. 1.55; $P = 0.005$). With adjustments applied, we found a borderline significant difference across the *ADD3* genotypes in the early peak of transmitral and lateral myocardial velocities (Table 3). For the mitral annular velocities and ratios at other sites ($P > 0.06$, data not shown) as well as the transmitral E/A ratio (Table 3), none of the phenotype–genotype associations reached statistical significance. Our findings on the lateral Ea/Aa ratio remained consistent after applying the square root transformation to the Ea/Aa distribution (Table 3).

Figure 2 shows the lateral mitral annular Ea, Aa, and Ea/Aa by age group and *ADD1* genotype. For lateral Ea and Ea/Aa, there was a significant interaction between the *ADD1* genotype and age analyzed as a continuous variable, indicating that the genetic effects of *ADD1* significantly weakened with older age. Table 4 shows *P* values for the simultaneous inclusion in models of *ADD1*, *ADD3*, and *ADD1* × *ADD3* and *ADD1* × *ADD3* × age interactions, along with other covariates. Although the *ADD1* × *ADD3* interaction was not significant ($P \geq 0.05$), the three-way interaction between the two genes and age improved the models ($P \leq 0.038$). After dichotomizing by mean age (50 years), the lateral Ea/Aa ratio, both before and after transformation, was higher in younger *ADD1 Trp* allele carriers compared with *GlyGly* homozygotes (1.93 vs. 1.73; $P = 0.012$ and 1.35 vs. 1.29; $P = 0.029$, respectively), particularly in the presence of *ADD3* GG homozygosity (2.46 vs. 1.80; $P = 0.0008$ and 1.53 vs. 1.33; $P = 0.0014$, respectively). In older subjects, these phenotype–genotype associations were not significant ($P > 0.20$). Specific and cumulative adjustments for the three classes of antihypertensive drugs as well as for 24-h sodium excretion did not alter our findings, as reported in Tables 3 and 4.

Table 2 Correlates of the transmitral and the lateral mitral annular velocities in stepwise regression

	Transmitral velocities			Lateral mitral annular velocities		
Parameter	*E* (m/s)	*A* (m/s)	*E/A*	Ea (cm/s)	Aa (cm/s)	Ea/Aa
R^2	0.317	0.612	0.690	0.659	0.443	0.694
Intercept	0.70 ± 0.083	0.30 ± 0.17	5.60 ± 0.17	24.4 ± 1.97	−8.53 ± 0.18	5.69 ± 0.20
Partial regression coefficients						
Age (>10 years)	−0.063 ± 0.006‡	0.057 ± 0.005 ±	−0.238 ± 0.010‡	−1.825 ± 0.094‡	0.958 ± 0.073‡	−0.351 ± 0.014‡
Woman (0,1)	−0.094 ± 0.013‡	−0.031 ± 0.015*	–	–	–	–
Body mass index (>1 kg/m^2)	–	0.006 ± 0.002‡	−0.015 ± 0.004‡	−0.214 ± 0.033‡	0.064 ± 0.025†	−0.033 ± 0.005‡
Heart rate (>10 beats/min)	−0.040 ± 0.007‡	0.039 ± 0.006‡	−0.14 ± 0.014‡	–	0.877 ± 0.098‡	−0.142 ± 0.020‡
Systolic BP (>10 mmHg)	0.010 ± 0.004*	0.020 ± 0.004‡	–	–	–	–
Diastolic BP (>10 mmHg)	–	–	−0.059 ± 0.016‡	−0.656 ± 0.141‡		−0.096 ± 0.024‡
LV length (>1 cm)	–	–	–	0.784 ± 0.157‡	0.659 ± 0.127‡	–
Ejection fraction (>10%)	0.051 ± 0.009‡	–	0.082 ± 0.019‡	0.349 ± 0.162*	0.310 ± 0.127*	–

Values are mutually adjusted partial regression coefficients ±SD. BP and LV indicate blood pressure and left ventricle, respectively. * Significance of the partial regression coefficients: $P \leq 0.05$. † Significance of the partial regression coefficients: $P \leq 0.01$. ‡ Significance of the partial regression coefficients: $P \leq 0.001$.

Family-based association study

In 117 sib–sib pairs, the multivariate-adjusted intrafamilial correlation coefficients for the averaged Ea, Aa, and Ea/Aa were 0.44, 0.37, and 0.43, respectively ($P < 0.001$ for all). The correlation coefficients for the corresponding transmitral measurements were 0.29 ($P = 0.003$), 0.09 ($P = 0.37$), and 0.20 ($P = 0.045$).

Our family-based analyses included 29 complex families and 332 offspring (mean age 46.9 ± 14.4 years; 49% women). Of 29 complex families, 10, 13, and 6 spanned 1, 2, and 3 generations, respectively. The lateral Ea and the Ea/Aa ratio, both before and after transformation, increased with transmission of the *ADD1 Trp* allele. The effect sizes averaged +0.91 ± 0.40 ($P = 0.026$), +0.23 ± 0.067 ($P = 0.0008$), and +0.075 ± 0.024 ($P = 0.002$), respectively. The effect sizes associated with transmission of the *ADD3 G* allele were −0.050 ± 0.34 ($P = 0.88$), +0.013 ± 0.059 ($P = 0.83$), and −0.0043 ± 0.022 ($P = 0.90$) for the lateral Ea and the untransformed and transformed lateral Ea/Aa ratios, respectively. In all models, the between-family component of variation in the lateral Ea and Ea/Aa ratio, both before and after transformation, was not statistically significant ($P > 0.09$). This was also the case for the *ADD1* × *ADD3* interaction terms ($P > 0.11$).

Discussion

With adjustments applied for age, body mass index, heart rate, and diastolic blood pressure, the lateral mitral annular Ea/Aa ratio was higher in *ADD1 Trp* allele carriers than in *GlyGly* homozygotes and lower in *ADD3 A* allele carriers than in *GG* homozygotes. Age weakened the association of the lateral Ea and Ea/Aa with *ADD1* (Fig. 1). The best fitting model for the lateral Ea, Ea/Aa, and square root of the Ea/Aa ratio included *ADD1*, *ADD3*, and the three-way interaction term of both genes with age. Transmission of the *ADD1 Trp* allele to offspring was associated with higher lateral Ea, Ea/Aa ratio, and square root of the Ea/Aa ratio.

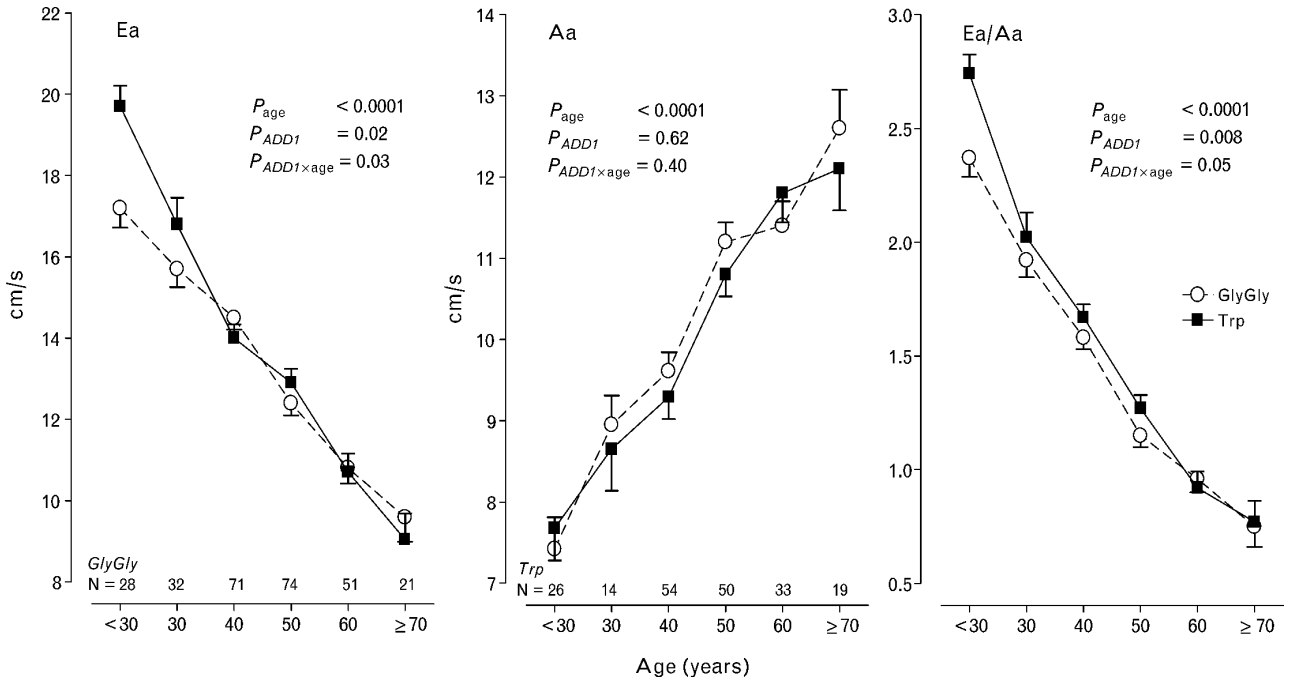
We assessed left ventricular diastolic function using the transmitral flow and the TDI mitral annular velocities. Lower transmitral E/A ratio and mitral annular Ea/Aa ratio both reflect impaired myocardial relaxation, characterized by early decreased but enhanced atrial filling of the left ventricle. The Ea peak velocity along the left ventricular longitudinal axis, however, is less susceptible to the effects of an increased preload and therefore provides a more direct measure of myocardial relaxation than does the transmitral E peak velocity [12,13]. Indeed, TDI recordings reflect the change in the left ventricular relaxation in

Table 3 Transmitral and lateral mitral annular velocities and ratios by genotypes in single-gene analyses

	ADD1			*ADD3*			
Velocity	GlyGly ($n = 277$)	Trp carriers ($n = 196$)	*P*	AA ($n = 138$)	AG ($n = 220$)	GG ($n = 115$)	*P*
Transmitral velocities							
E (cm/s)	77.1 ± 0.88	77.4 ± 1.01	0.85	78.1 ± 1.12	75.3 ± 0.90	79.1 ± 1.23	0.025
A (cm/s)	64.3 ± 0.74	64.4 ± 0.85	0.94	65.3 ± 0.98	63.6 ± 0.80	64.8 ± 1.10	0.34
E/A	1.30 ± 0.018	1.30 ± 0.021	0.92	1.30 ± 0.024	1.28 ± 0.020	1.33 ± 0.028	0.27
Lateral mitral annular velocities							
Ea (cm/s)	13.5 ± 0.17	13.8 ± 0.19	0.23	13.7 ± 0.22	13.3 ± 0.19	14.1 ± 0.25	0.028
Aa (cm/s)	10.2 ± 0.13	10.0 ± 0.15	0.22	10.0 ± 0.18	10.3 ± 0.15	9.97 ± 0.20	0.29
Ea/Aa	1.40 ± 0.027	1.51 ± 0.031	0.005	1.46 ± 0.037	1.40 ± 0.030	1.55 ± 0.042	0.007
Square root of Ea/Aa	1.14 ± 0.010	1.19 ± 0.012	0.009	1.16 ± 0.013	1.14 ± 0.011	1.20 ± 0.015	0.027

Values are least square means ± SD adjusted for family clusters, age, body mass index, heart rate, and supine diastolic blood pressure measured immediately after echocardiography. Velocities were additionally adjusted for ejection fraction and left ventricular length. *P* values are for the differences between *ADD1 GlyGly* homozygotes and *Trp* allele carriers and across the *ADD3* genotypes.

Fig. 2



Lateral mitral annular Ea, Aa, and Ea/Aa ratio by age group and *ADD1* genotype

Plotted values were adjusted for body mass index, heart rate, and diastolic blood pressure. *N* indicates the number of *GlyGly* homozygotes (open symbols) and *Trp* allele carriers (closed symbols) contributing to the plotted least square means. *P* values are for the effects of age (P_{age}), *ADD1* (P_{ADD1}), and the interaction term between *ADD1* and age analyzed as a continuous variable ($P_{ADD1 \times age}$).

the long-axis dimension [13]. In keeping with this concept, we found that left ventricular volumes affected the transmitral E/A ratio but not the mitral annular Ea/Aa ratio. This observation might explain why among offspring we could demonstrate highly significant heritability for the Ea/Aa ratio but only borderline significant heritability for the E/A ratio. Similarly, in a study of hypertensive patients, the treatment-induced changes in the Ea/Aa ratio were more significant than those in the E/A ratio [14].

In keeping with previous studies on patients and healthy subjects [15,16], we noticed that the mitral annular velocities were greater at the lateral site than at other locations, in particular, the interventricular septum. This is the physiologic consequence of the influence of the right ventricle and the anatomic arrangement of myocardial fibers [17]. Although long-axis fibers are mainly present in the subendocardial and subepicardial layers of the left ventricular free wall, they are almost absent in the septum. Kasner *et al.* [18] recently found that the lateral annular velocities were more closely related to the left ventricular relaxation and compliance indexes, as determined by invasive pressure–volume loops, than to the septal annular velocities. Using this sensitive measure, we found a significantly higher lateral Ea/Aa ratio in *ADD1 Trp* allele carriers compared with *GlyGly* homozygotes and in *ADD3 GG* homozygotes compared with *A* allele carriers.

Table 4 Multivariate-adjusted models of mitral annular velocities with genetic variation in *ADD1* and *ADD3*

		Mitral annular velocities					
	df	Lateral Ea		Lateral Ea/Aa		Square root of lateral Ea/Aa	
Covariates added to the basic model		*F* value	*P*	*F* value	*P*	*F* value	*P*
ADD1	1	6.01	0.022	14.0	0.001	7.26	0.013
ADD3	2	4.98	0.011	12.8	<0.0001	8.48	0.0008
ADD1 × *ADD3* interaction	2	0.51	0.76	5.04	0.05	3.03	0.12
ADD1 × *ADD3* × age interaction	5	2.37	0.038	5.83	<0.0001	3.50	0.004

df indicates degrees of freedom. *P* values are for the simultaneous inclusion in the models of *ADD1*, *ADD3*, and the *ADD1* × *ADD3* and *ADD1* × *ADD3* × age interactions. All models also accounted for age, body mass index, heart rate, and diastolic blood pressure. For the lateral Ea, the model additionally included ejection fraction and left ventricular length.

Previous studies on populations and patients have demonstrated that the *ADD1 Gly460Trp* polymorphism, alone or in combination with variation in the *ADD3* (*IVS11 +386A>G*) or *ACE* (*I/D*) genes, influences the peripheral and central blood pressure [19], the daytime and nighttime ambulatory blood pressure [20], the prevalence and incidence of hypertension [7], femoral intima–media thickness [21], the distensibility of the large arteries [22], and the risk of cardiovascular events [8,23]. In never-treated hypertensive patients of Italian origin [24], the absolute and relative changes in mean blood pressure in response to acute sodium loading depended on the epistatic interaction between *ADD1* and *ADD3*. Indeed, mean arterial pressure increased to the largest extent in patients carrying both the mutated *ADD1 Trp* allele and the *ADD3 GG* genotype. The epistatic interaction between the *ADD1* and *ADD3* genes, which are located on different chromosomes, is in keeping with the heterodimeric structure of the adducin protein and strengthens the role of these genes compared with that of other loci mapping near to the adducin subunits.

The mechanisms underlying the association of the rate of myocardial relaxation with genetic variation in *ADD1* and *ADD3* remain to be elucidated. Mutation of *ADD1* is associated with increased Na^+,K^+-ATPase activity [2,3] and increased renal tubular sodium reabsorption [4]. It is conceivable that the constitutive activation of the sodium pump in *ADD1 Trp* allele carriers not only occurs in renal tubular cells but might also be present in cardiomyocytes. Na^+,K^+-ATPase activity mediates Na^+ efflux [25]. Ca^{2+} extrusion in cardiomyocytes depends almost completely on the NCX system, which colocalizes with the sodium pump in the sarcolemmal membrane [25]. Small perturbations in the intracellular Na^+ concentration can greatly influence Ca^{2+} fluxes [6]. Thus, substitution of glycine by tryptophan in the α-subunit of adducin might lead to enhanced Na^+,K^+-ATPase activity and lower intracellular Na^+ concentration in cardiomyocytes. In turn, this mechanism might entail a faster Ca^{2+} efflux through NCX during diastole and therefore facilitate myocardial relaxation. We did not find any argument supporting the idea that the enhanced renal sodium reabsorption in carriers of the *ADD1 Trp* allele [4] might explain the currently observed genetic association with myocardial relaxation. Indeed, adjustment for ejection fraction did not remove the genetic association. Moreover, there were no differences between the genotypes under study in the atrial end-systolic and the left ventricular end-diastolic volumes (data not shown). We cannot exclude the possibility, however, that a long-lasting moderate expansion of blood volume might have contributed to the current observations.

In line with the literature, left ventricular diastolic function decreased with older age. In older subjects, chronic age-related disorders, such as hypertension and obesity, and the life-long exposure to confounding factors related to lifestyle and environment might weaken the association of left ventricular diastolic function with the adducin genotypes. Moreover, changes in the transmembranous Ca^{2+} fluxes might be related to faster left ventricular relaxation at young age and susceptibility to heart failure at old age. In *ADD1 Trp* allele carriers, there might be a decrease in the intracardiomyocyte Ca^{2+} concentration, which might affect the excitation–contraction coupling and might not be beneficial in the long run. In line with this hypothesis, we previously observed that the *ADD1 Trp* allele was associated with a significantly higher incidence of heart failure [8]. Nevertheless, further experimental studies should elucidate whether the Ca^{2+} concentration in cardiomyocytes is indeed lower in carriers of the mutated *ADD1*.

The present study must be interpreted within the context of its limitations and strengths. We measured only one SNP per gene. We might, therefore, have underestimated the full functional impact of the adducin genes on left ventricular diastolic function. Nevertheless, a large number of experimental and clinical studies have documented the functionality of the *ADD1 Gly460Trp* polymorphism [4]. The *A*-to-*G* substitution in *ADD3* is located in intron 11 (*IVS11 +386A>G* –rs3731566). Neither previous publications nor genome browser databases provided any suggestion about its functional role. A nucleotide variation analysis is needed to elucidate the pattern of linkage disequilibrium of the entire *ADD3* locus and establish whether this intronic common polymorphism is linked to the 'causal' SNP or is a regulatory variant 'per se'. According to the latter hypothesis, preliminary data show that *ADD3* mRNA level is significantly enhanced in *GG* homozygotes compared with the other genotypes in 39 kidney cortex samples from human donors (Lorena Citterio, Milan, Italy, personal communication). Transmitral and TDI velocities are quantitative traits that arise from complex interaction between multiple genes, hemodynamic, and environmental factors and are prone to measurement error. In the present study, one experienced observer performed all echocardiograms, with high inter-observer reproducibility. There was also a high degree of internal consistency between the results of the population-based and family-based analyses. The between-family component of the quantitative transmission disequilibrium test was not statistically significant, which makes it unlikely that our results are driven by population stratification.

In conclusion, our population-based study demonstrated that left ventricular diastolic relaxation is modulated by genetic variation in *ADD1* and *ADD3*. This association was more prominent in younger subjects in whom long-standing environmental factors and ageing are less likely to mask genetic effects. Whether changes in the Na^+ and

Ca^{2+} concentrations in cardiomyocytes might explain our current findings remains to be clarified.

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References

1 Matsuoka Y, Li X, Bennett V. Adducin: structure, function and regulation. *Cell Mol Life Sci* 2000; **57**:884–895.
2 Tripodi G, Valtorta F, Torielli L, Chieregatti E, Salardi S, Trusolino L, *et al.* Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport. *J Clin Invest* 1996; **97**:2815–2822.
3 Efendiev R, Krmar RT, Ogimoto G, Zwiller J, Tripodi G, Katz AI, *et al.* Hypertension-linked mutation in the adducin α-subunit leads to higher AP2-μ2 phosphorylation and impaired Na^+,K^+-ATPase trafficking in response to GPCR signals and intracellular sodium. *Circ Res* 2004; **45**:331–340.
4 Bianchi G, Ferrari P, Staessen JA. Adducin polymorphism. Detection and impact on hypertension and related disorders. *Hypertension* 2005; **45**:331–340.
5 Citterio L, Tizzoni L, Catalano M, Zerbini G, Bianchi G, Barlassina C. Expression analysis of the human adducin gene family and evidence of ADD2 β4 multiple splicing variants. *Biochem Biophys Res Commun* 2003; **309**:359–367.
6 Blaustein MP, Lederer WJ. Sodium/calcium exchange: its physiological implications. *Physiol Rev* 1999; **79**:763–854.
7 Staessen JA, Wang JG, Brand E, Barlassina C, Birkenhäger WH, Herrmann SM, *et al.* Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *J Hypertens* 2001; **19**:1349–1358.
8 Li Y, Zagato L, Kuznetsova T, Tripodi G, Zerbini G, Richart T, *et al.* Angiotensin-converting enzyme *I/D* and α-adducin *Gly460Trp* polymorphisms. From angiotensin-converting enzyme activity to cardiovascular outcome. *Hypertension* 2007; **49**:1291–1297.
9 El Assaad MA, Topouchian JA, Asmar RG. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. *Blood Press Monit* 2003; **8**:127–133.
10 Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, *et al.*, American Society of Echocardiography recommendations for use of echocardiography in clinical trials. A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Task Force on Echocardiography in Clinical Trials. *J Am Soc Echocardiogr* 2004; **17**:1086–1119.
11 Abecasis GR, Cardon LR, Cookson WOC. A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000; **66**:279–292.
12 Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quiñones MA. Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *J Am Coll Cardiol* 1997; **30**:1527–1533.
13 Garcia MJ, Thomas JD, Klein AL. New Doppler echocardiographic applications for the study of diastolic function. *J Am Coll Cardiol* 1998; **32**:865–875.
14 Di Bello V, Giorgi D, Pedrinelli R, Talini E, Palagi C, Delle Donne MG, *et al.* Left ventricular hypertrophy and its regression in essential arterial hypertension. A tissue Doppler imaging study. *Am J Hypertens* 2004; **17**:882–890.
15 Alam M, Wardell J, Andersson E, Samad BA, Nordlander R. Characteristics of mitral and tricuspidal annular velocities determined by pulsed wave Doppler tissue imaging in healthy subjects. *J Am Soc Echocardiogr* 1999; **12**:618–626.
16 Palecek T, Linhart A. Comparison of early diastolic annular velocities measured at various sites of mitral annulus in detection of mild to moderate left ventricular diastolic dysfunction. *Heart Vessels* 2007; **22**:67–72.
17 Greenbaum RA, Ho SY, Gibson DG, Becker AE, Anderson RH. Left ventricle fibre architecture in man. *Br Heart J* 1981; **45**:248–258.
18 Kasner M, Westermann D, Steendijk P, Gaub R, Wilkenshoff U, Weitmann K, *et al.* Utility of Doppler echocardiography and tissue Doppler imaging in the estimation of diastolic function in heart failure with normal ejection fraction. *Circulation* 2007; **116**:637–647.
19 Cwynar M, Staessen JA, Tichá M, Nawrot T, Citterio L, Kuznetsova T, *et al.*, On behalf of the European Project on Genes in Hypertension (EPOGH) Investigators. Epistatic interaction between α- and γ-adducin influences peripheral and central pulse pressure in White Europeans. *J Hypertens* 2005; **23**:961–969.
20 Lanzani C, Citterio L, Jankaricova M, Sciarrone MT, Barlassina C, Fattori S, *et al.* Role of the adducin family genes in human essential hypertension. *J Hypertens* 2005; **23**:543–549.
21 Balkestein EJ, Wang JG, Struijker Boudier HAJ, Barlassina C, Bianchi G, Birkenhäger WH, *et al.* Carotid and femoral intima–media thickness in relation to three candidate genes in a Caucasian population. *J Hypertens* 2002; **20**:1551–1561.
22 Balkestein EJ, Staessen JA, Wang JG, van der Heijden-Spek JJ, Van Bortel L, Barlassina C, *et al.* Carotid and femoral artery stiffness in relation to three candidate genes in a white population. *Hypertension* 2001; **38**:1190–1197.
23 Li Y, Thijs L, Kuznetsova T, Zagato L, Struijker-Boudier H, Bianchi G, Staessen JA. Cardiovascular risk in relation to α-adducin *Gly460Trp* polymorphism and systolic pressure. A prospective population study. *Hypertension* 2005; **46**:527–532.
24 Manunta P, Lanzani C, Sciarrone MT, Bracale M, Redaelli A, Citterio L, *et al.* Modulation of the pressure–natriuresis relationship by ADD1 and ADD3 polymorphisms. *Hypertension* 2004; **44**:578 [abstract].
25 Bers DM. Altered cardiac myocyte Ca regulation in heart failure. *Physiology* 2006; **21**:380–387.

Candidate gene studies in cardiovascular medicine: complex diseases and even more complex intermediate phenotypes

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Genetic factors account for about 30% of blood pressure variation in humans [1]. A large number of models and techniques ranging from rodent models of hypertension [2,3] to genome-wide association studies [4,5] are available to dissect these genetic factors. However, there has only been limited success in the hunt for human blood pressure genes. Fortunately enough, there are some success stories. In this issue of *Journal of Hypertension*, we read a report on another piece of the genetic jigsaw of hypertension and related cardiovascular disease. Kuznetsova *et al.* [6] found an association between variants of adducin genes and left ventricular diastolic function, a potential early step in the development of heart failure.

Role of adducin in cardiovascular disease

Adducin genes are among the most promising functional candidate genes for hypertension and hypertension-related organ damage. Adducin is a heterodimeric cytoskeleton protein that consists of an α-subunit encoded by the *ADD1* gene and either a β-subunit or a γ-subunit encoded by *ADD2* and *ADD3*, respectively [2,7]. Adducin is involved in the regulation of Na^+/K^+ ATPase activity particularly in renal tubular cells. Mutations in *ADD1* lead to increased renal sodium reabsorption, volume expansion and, ultimately, hypertension [2]. Alteration in renal sodium handling is a major pathogenetic mechanism of hypertension [8], and adducin is placed right in the center of this mechanism. Being of crucial importance for cellular homeostasis, it is not surprising that adducin genes are highly conserved across species [7], which in turn increases the likelihood for mutations in adducin genes being of functional relevance; a large body of evidence supports this notion. Mutations of adducin genes have been found to be associated with the slope of the pressure–natriuresis curve [9], hypertension [10] and heart failure [11]. In line with the putative pathophysiological role of adducin variants, the association between *ADD1* genotypes and blood pressure is modified by salt intake [12], and *ADD1* genotype determines the response of blood pressure to thiazide diuretics [13].

The present study by Kuznetsova *et al.* [6] examines a different aspect of the role of adducin variants in cardiovascular disease. *ADD1* and *ADD3* are also expressed in cardiac tissue [14] and it is therefore a logical step to examine the relationship between genetic variants of these genes and cardiac function in more detail. The authors examined the *ADD1* Gly460Trp and *ADD3* A386G polymorphisms in the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) and found increased lateral Ea/Aa ratio in Trp carriers of the *ADD1* variant and reduced lateral Ea/Aa ratio in carriers of the A allele of the *ADD3* variant. In a family-based approach, transmission of the *ADD1* Trp allele was associated with increased Ea/Aa ratio. These findings have to be viewed within the context of the previously reported association of the *ADD1* Trp allele with heart failure and may contribute to the modulating effect of *ADD1* genotype on the association between blood pressure and cardiovascular outcome [11].

The present study [6] provides evidence that adducin gene variants are involved in each step of the continuum from hypertension to heart failure. This is probably not surprising. As outlined by Bianchi [2], detecting genotype–phenotype associations for complex phenotypes on the level of the whole body is more difficult than detecting associations with intermediate phenotypes. In the case of *ADD1*, we already know that a genetic variant is associated with heart failure [11] and therefore an association with a very early step in the development of heart failure should have been expected. It still had to be proven and, in that sense, the present study is a small but important step forward.

Detailed phenotyping and adjustment for covariates

The study by Kuznetsova *et al.* [6] is characterized by its exemplary accurate phenotyping. The investigators used tissue Doppler imaging, which overcomes some of the weaknesses of standard Doppler echocardiography [15,16]. Tissue Doppler imaging enabled the authors to examine lateral mitral annular velocities. A more sensitive and specific lateral Ea/Aa ratio was required to detect the reported genotype–phenotype relationships. Especially in a general population cohort that is not enriched for presence of cardiovascular disease, such accurate phenotyping is useful. Probably of even greater

importance than the actual technique, this study benefits from the fact that all echocardiographic assessments have been carried out by a single experienced examiner. Performing about 500 echocardiograms is not a trivial task, but the results obtained were definitely worth the effort.

Looking at the results more closely, one has to admit that the differences in Ea/Aa across the genotypes are marginal. Moreover, participants examined in the study by Kuznetsova *et al.* [6] had entirely normal E/A ratio, and in a clinical setting, one would expect additional information from tissue Doppler imaging only in severe diastolic dysfunction with pseudo-normalization of E/A ratio [15]. The vast majority of study participants did certainly not have severe diastolic dysfunction and it remains to be proven that assessment of Ea/Aa is a useful intermediate phenotype in this scenario. It is also not entirely clear why participants with the best established risk allele (*ADD1* Trp allele) exhibit increased Ea/Aa ratio, which is better diastolic relaxation, compared with participants with the other allele. Basically, and this is what the authors stated correctly, the present study [6] shows a modulating effect of adducin genotypes on a marker of diastolic left ventricular function, but the mechanisms and relevance of this association need to be elucidated.

Another feature of the present study [6] is the adjustment for age. This adjustment was again necessary due to recruitment of participants from the general population with a wide age range, but it also led to a finding that is interesting on its own. The fact that the association between *ADD1* and *ADD3* genotypes and left ventricular diastolic function was only present in younger people shows an important interaction between genotype and confounding factors such as age. Age and long-term exposure to cardiovascular risk factors including hypertension is a so much stronger determinant of reduced left ventricular diastolic function that the relatively small effect of *ADD1* and *ADD3* genotype was not detectable in elderly people. This finding reminds us that patient selection and definition of inclusion and exclusion criteria in genetic association studies are as important as in any other clinical study. It probably also shows that a general population sample with a wide age range, an average normal blood pressure and normal left ventricular function is not particularly suited to dissect the relationship between adducin genotype and cardiac function. Kuznetsova *et al.* compensated for this by their excellent phenotyping, but a strategy to enrich a cohort for the phenotype under investigation appears preferable for many genetic studies [4,5].

The challenge of a candidate gene study in 2008 and lessons from the past

Strengths and weaknesses of genetic association studies have been previously addressed in editorial commentaries in this journal [17,18]. Sharma and Jeunemaitre [17] noted in 2000 that 'the more extensive the phenotypic data available, the higher the chance of finding a spurious association with some aspect of the phenotype'. This statement still applies to 2008 and shows that although generally being a blessing, the detailed phenotyping in the study by Kuznetsova *et al.* [6] can also be a curse. Being the devil's advocate, one could argue that the authors simply reported a spurious association of a specific phenotype with two genetic variants and disregarded other potentially nonsignificant results. However, the reason for examining this specific phenotype, namely Ea/Aa ratio, is well justified and builds upon a series of previous association studies and pathophysiological considerations. In that sense, we may trust that the present findings are indeed sound and meaningful. However, in order to avoid misinterpretation, Sharma and Jeunemaitre [17] recommend that '[a] clear a priori hypothesis should be stated in the study design'. This is certainly excellent advice, but given the expenses of both genotyping and phenotyping, it is unlikely that large cohorts will be recruited in the future with the aim of examining only a limited range of questions. Indeed, especially the large patient cohorts that have been collected in recent years are currently being used for a number of secondary analyses and are even restratified and combined to create new cohorts to facilitate testing of specific hypotheses. The FLEMENGHO study is an example for a study that has been used to answer a number of different questions in the past and, in line with the notion by Sharma and Jeunemaitre [17], one should probably not overinterpret the present results by Kuznetsova *et al.* [6].

Statistical problems with multiple tests are obvious in studies reporting a huge number of tests at a time, notably in the current genome-wide association studies [4,5]. Multiple testing is hidden in studies that have been used repeatedly to examine different aspects of disease and different genetic variants. This has been previously noted in a commentary by Hilgers and Schmieder in 2002 [18] and the situation remains the same in 2008. Even worse, in addition to the above-mentioned diversification of phenotypes, we are now also in a position of having data on hundreds of thousands of genetic variations at relatively low cost. This further increases the number of possible genetic association studies in cohorts that have been recruited previously. Kuznetsova *et al.* [6] will have to provide evidence that the observed association holds true in independent cohorts and deliver functional data on the genetic variants under study. To be fair, the observation that *ADD1* genotype is associated with Ea/Aa ratio, not only in a population-based approach but also in a family-based approach in the present study, adds substantial value to the findings. Also, the briefly mentioned data on renal *ADD3* expression are a step in the right direction, but ultimately one would like to see

data on *ADD1* and *ADD3* expression in myocardial tissue and electrophysiological studies.

The future of candidate gene studies in cardiovascular disease

In this era of genome-wide association studies, it is refreshing to read a straightforward candidate gene study such as that by Kuznetsova *et al.* [6], especially if it benefits from state-of-the-art phenotyping. It is unfortunate that genome-wide association studies for hypertension have not yet come up with significant results even for promising candidate genes such as *ADD1* and *ADD3* [4,5]. This probably shows that future strategies to unravel the genetics of cardiovascular disease, and in particular hypertension, will depend on both genome scans and candidate gene studies that complement each other. However, candidate gene studies and particularly candidate gene studies from well renowned research groups would benefit from recent developments in genotyping technologies offering a far more extensive characterization of genetic variation compared with one or two single nucleotide polymorphisms. They would also benefit from adding functional data and repetition of results in independent cohorts [19]. With regard to the study by Kuznetsova *et al.* [6], it is likely that present findings will eventually be confirmed. We look forward to repetition studies in different cohorts and to functional data in the near future.

References

1 Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. *Lancet* 2003; **361**:1629–1641.
2 Bianchi G. Genetic variations of tubular sodium reabsorption leading to 'primary' hypertension: from gene polymorphism to clinical symptoms. *Am J Physiol Regul Integr Comp Physiol* 2005; **289**:R1536–R1549.
3 Rapp JP. Genetic analysis of inherited hypertension in the rat. *Physiol Rev* 2000; **80**:135–172.
4 Caulfield M, Munroe P, Pembroke J, Samani N, Dominiczak A, Brown M, *et al.* Genome-wide mapping of human loci for essential hypertension. *Lancet* 2003; **361**:2118–2123.
5 Wellcome Trust Case Control Consortium. Genome-wide association study of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; **447**:661–678.
6 Kuznetsova T, Citterio L, Herbots L, Delli Carpini S, Thijs L, Casamassima N, *et al.* Effects of genetic variation in adducing on left ventricular diastolic function assessed by Tissue Doppler Imaging in a Flemish population. *J Hypertens* 2008; **26**:1229–1236.
7 Matsuoka Y, Li X, Bennett V. Adducin: structure, function and regulation. *Cell Mol Life Sci* 2000; **57**:884–895.
8 Johnson RJ, Feig DI, Nakagawa T, Sanchez-Lozada LG, Rodriguez-Iturbe B. Pathogenesis of essential hypertension: historical paradigms and modern insights. *J Hypertens* 2008; **26**:381–391.
9 Manunta P, Cusi D, Barlassina C, Righetti M, Lanzani C, D'Amico M, *et al.* Alpha-adducin polymorphisms and renal sodium handling in essential hypertensive patients. *Kidney Int* 1998; **53**:1471–1478.
10 Staessen JA, Wang JG, Brand E, Barlassina C, Birkenhäger WH, Herrmann SM, *et al.* Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *J Hypertens* 2001; **19**:1349–1358 [erratum in J Hypertens 2001; 19:1921].
11 Li Y, Zagato L, Kuznetsova T, Tripodi G, Zerbini G, Richart T, *et al.* Angiotensin-converting enzyme I/D and alpha-adducin Gly460Trp polymorphisms: from angiotensin-converting enzyme activity to cardiovascular outcome. *Hypertension* 2007; **49**:1291–1297.
12 Kuznetsova T, Staessen JA, Brand E, Cwynar M, Stolarz K, Thijs L, *et al.* Sodium excretion as a modulator of genetic associations with cardiovascular phenotypes in the European Project on Genes in Hypertension. *J Hypertens* 2006; **24**:235–242.
13 Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, Bianchi G, Cusi D. ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. *Hypertension* 2003; **41**:398–403.
14 Citterio L, Tizzoni L, Catalano M, Zerbini G, Bianchi G, Barlassina C. Expression analysis of the human adducin gene family and evidence of ADD2 beta4 multiple splicing variants. *Biochem Biophys Res Commun* 2003; **309**:359–567.
15 Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quiñones MA. Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *J Am Coll Cardiol* 1997; **30**:1527–1533.
16 Garcia MJ, Thomas JD, Klein AL. New Doppler echocardiographic applications for the study of diastolic function. *J Am Coll Cardiol* 1998; **32**:865–875.
17 Sharma AM, Jeunemaitre X. The future of genetic association studies in hypertension: improving the signal-to-noise ratio. *J Hypertens* 2000; **18**:811–814.
18 Hilgers KF, Schmieder RE. Association studies in cardiovascular medicine. *J Hypertens* 2002; **20**:173–176.
19 Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, *et al.*, NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype–phenotype associations. *Nature* 2007; **447**:655–660.