Heritability of left ventricular and papillary muscle heart size: a twin study with cardiac magnetic resonance imaging

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Aims
Earlier studies in monozygotic (MZ) and dizygotic (DZ) twins showed genetic variance on echocardiographically determined heart size. However, cardiovascular magnetic resonance (CMR) is more precise and reproducible. We performed a twin study relying on CMR, focusing on left ventricular (LV) mass and papillary muscle, since there are no genetic reports on this structure.

Methods and results
We measured left heart dimensions of 25 healthy twin pairs with a 1.5T MR scanner, analysed with the mass©, Medis Software. We performed heritability analysis and tests for genetic influences shared between cardiac structures. We found that CMR-based heritability estimates ($h^2 = 84\%$) substantially exceeded estimates based on echocardiography. We also found significant genetic influence on papillary muscle mass ($h^2 = 82\%$). Bivariate analysis of papillary and LV muscle mass revealed significant genetic influences shared by both phenotypes (genetic correlation 0.59) and suggested an additional genetic component specific to papillary muscle. We observed correlations between body mass index, surface area, and systolic blood pressure with cardiac dimensions, even in this small study. Environmental influences were relevant as well, indicating reciprocal influences on papillary vs. LV muscle mass.

Conclusion
Cardiovascular magnetic resonance, even with few subjects, allows a genetic assessment of cardiac structures that cannot be attained with echocardiography. Hitherto unappreciated relationships can be uncovered by this method.

Keywords
Genetics • Heart • Twins • Magnetic resonance imaging • Heredity

Introduction
Monozygotic (MZ) and dizygotic (DZ) twins are a useful model to determine genetic variance. Adams et al.1 performed the first echocardiographic and electrocardiographic studies in young normal MZ and DZ twins, siblings of like sex, and non-related subjects and determined genetic variance on heart size. A smaller subgroup was subjected to 14 weeks of exercise. With the diagnostic techniques available at the time and the heterogeneity of subjects and protocol, the authors concluded that cultural familial influences are more important in determining cardiac size than non-familial or even genetic influences. In a previous study from our group, we used echocardiography to assess posterior wall thickness, septum thickness, and left ventricular (LV) mass index in 100 MZ pairs and 66 DZ pairs of normal young twins. We found robust evidence for genetic variance on septum thickness and LV mass, whereas posterior wall thickness heritability was less robust.2 Cardiac magnetic resonance (CMR) is a better method to quantitatively image the heart.3,4 Oddly, only a single case report of an MZ twin pair with hypertrophic cardiomyopathy has been published using CMR.5 No twin study has been performed to test the utility of CMR in determining genetic variance. We tested the utility of CMR to assess heritability estimates in MZ and DZ twins, expecting an increase in intra-pair correlation and heritability due to the reduction of measurement imprecision. Not only were we able to assess structures difficult to measure...
with echocardiography, but also we found robust associations with unexpected risk variables in a surprisingly low number of subjects.

**Methods**

We studied 25 twin pairs: 13 twin pairs were MZ (age 34 ± 10 years, 4 men and 22 women) and 12 pairs DZ (age 31 ± 8 years, six pairs female/male, two pairs male/male, four pairs female/female). Zygosity was determined using at least five microsatellite markers co-amplified by polymerase chain reaction. The probability of a DZ twin pair to share all marker alleles by chance is 0.006. The overall rate of correct classification is >99%. The twin pairs were recruited from the Berlin Twin Registry. We obtained a medical history and conducted a physical examination prior to study entry. Blood pressure was measured by experienced study nurses after a 5' rest; the average of three measurements was recorded. Only healthy persons were included in the study. Persons ingesting medications except birth control pills were excluded from the study. The study complies with the Declaration of Helsinki, our internal review board approved the study and written, informed consent was obtained from all participants.

All examinations were performed on a 1.5T MR scanner (CVi, GE Medical Systems, Milwaukee, WI, USA) using a dedicated cardiac phased array coil, prospective electrocardiographic gating with subjects in a supine position. Localization was performed using end-expiration breath hold real-time and steady-state free precession images of true anatomical axes of the heart (TE 4.4 ms, TR 12.1 ms, 10 mm contiguous slices, acquisition matrix 256 × 192, FOV 36 × 36 cm, 30 phases per RR interval).

A single operator (CAB) evaluated all images randomized and in a blinded fashion using dedicated software (MASS 6, Medis, Leiden, The Netherlands). Manual tracing of the endocardial and epicardial borders was performed in the end-systolic and end-diastolic frames of the short-axis views (Figure 1). We monitored the contour tracing by reviewing the movie with and without the contours. The most basal slice was selected for end-systole and -diastole, when the blood volume was surrounded by myocardium by at least 50%. The most apical slice was defined as the last slice with visible intra-cavity blood. Generally, the papillary muscles were included to the myocardium (referred to as overall LV mass). Left ventricular mass was calculated from the total myocardial volume multiplied by the specific gravity of the myocardium (1.05 g/mL). Stroke volume (SV) and ejection fraction (EF) were calculated as SV = end-diastolic volume (EDV) – end-systolic volume; EF = SV/EDV × 100%. In a second step, the estimation of the LV mass in end-diastole was repeated with exclusion of papillary muscles (referred to as LV wall mass). The difference between both measurements was considered as the mass of the papillary muscles (PMM). To estimate inter-observer variation, a second highly experienced observer (HAA) analysed a total of 21 scans. For intra-observer variation, the observer (CAB) evaluated 10 scans twice.

Statistical analysis was conducted using the SPSS program. A value of \( P < 0.05 \) was considered to be statistically significant. All data are expressed as mean ± SD. Relationship between parameters was assessed by correlation analysis. Differences of mean group parameters were tested with unpaired t-tests. To test the relationship of overall LV mass, LV wall mass, and papillary muscle mass to known potential risk factors (blood pressure and lipids), we employed a correlation analysis. Parameters of the quantitative genetic models were estimated by structural equation modelling using the Mx program developed by Neale et al. The variability of any given phenotype within a population can be decomposed into additive genetic influences (Var_addGen, A), common environmental influences shared by the twins within a family (Var_comEnv, C), and effects of random environment (Var Env, E), this approach is called the ACE model:

\[
\text{Var} = \text{Var}_\text{addGen} + \text{Var}_\text{comEnv} + \text{Var}_\text{Env}
\]

For MZ and DZ, the covariance of their phenotype is given by:

\[
\text{Cov}_{\text{MZ}} = \text{Var}_\text{addGen} + \text{Var}_\text{comEnv}
\]
\[
\text{Cov}_{\text{DZ}} = 0.5\text{Var}_\text{addGen} + \text{Var}_\text{comEnv}
\]

Heritability analysis in twin studies can estimate genetic variability as well as two environmental influences, shared and non-shared environmental influences. These values estimate the relative amount of the variable’s influence on inter-individual differences up to a sum of one. No distinction between additive and dominant genetic factors was made in this study, as a general proof of genetic variance rather than a precise estimate of such influences was the goal of this study. Accordingly, broad-sense heritability is reported only; based on the single estimates for the sum of potential additive and non-additive genetic influences. As the power to detect environmental effects shared within twin pairs was limited and studies in larger cohorts did not find any evidence for such familial influences, we decided to generally drop common environment from the ACE model. Genetic as well as environmental effects were estimated by the best fitting model as selected by the \( \chi^2 \)-value. Nested models were tested, starting with the AE model, and then A was constrained to zero. The significance of a model component is tested by the drop-of-fit as measured by the difference in \( \chi^2 \). Adjustments for
age and sex were done by multiple linear regression using non-standardized residuals. To test if phenotypic correlations between different measures (LV mass and papillary muscle mass) are due to common underlying genetic or environmental factors, genetic correlations were computed on the basis of estimates of genetic covariance from a bivariate Cholesky model. The significance of genetic or environmental covariance was tested by the drop of model fit in a sub-model constraining the respective estimate to zero. Genetic correlation is defined by:

\[
\text{CorrGen} = \frac{\text{CovGen1Gen2}}{\sqrt{\text{VarGen1} \times \text{VarGen2}}}
\]

As in conventional correlation analysis, genetic correlation may vary between −1 and 1. It defines the sub-fraction of the genetic influence that is shared between traits, thus it can be greater than the heritability of either trait. Phenotypic correlations between cardiac dimensions and other phenotypes as for example body mass index (BMI) or blood pressure were analysed using partial correlations controlling for sex.

## Results

Demographic data and mean ± SD for cardiovascular data are given in Table 1. All values were within the normal range. There were no significant differences between MZ and DZ twins. LV mass exhibited an inter-observer intraclass correlation (ICC) of 0.88, whereas the intra-observer ICC was 0.99. We conclude from these results that environmental variance due to measurement error was negligible. As expected, most correlations within MZ pairs exceeded DZ similarity, as shown in Table 2. For LV mass, heritability estimates based on end-systolic, end-diastolic, and averaged measurements were similar \((h^2 = 0.82, 0.83, \text{ and } 0.84, \text{ respectively})\). The genetic influence on papillary muscle size itself was in the same range \((h^2 = 0.82)\) (Figure 2), whereas heritability of LV mass decreased to 0.69 when excluding papillary muscles. In addition to cardiac mass, we examined end-diastolic and end-systolic volumes. The heritability estimates were 0.76 and 0.27, respectively.

The phenotypic correlation within individuals between LV mass and papillary muscle mass was 0.56, indicating that genetic and/or environmental influences may have different effects on the two types of cardiac muscle. Bivariate analysis of papillary and LV mass revealed significant genetic influences shared by both phenotypes with a genetic correlation of 0.59 (Figure 3). Aside from this common genetic influence, there was an additional significant genetic component specific to papillary muscle. Environmental influences were shared by LV wall and papillary mass as well. The environmental correlation was −0.36, which suggests reciprocal influences.

We also performed correlation analysis between cardiac muscle mass and CMR-related measures that we adjusted for sex, shown in Table 3. We eschewed a comprehensive multivariate genetic analysis because of the small sample size. Nonetheless, we were interested to observe that BMI, body surface area, dry lean mass, and systolic blood pressure appeared in this model, as we would have expected from our earlier study. Systolic blood pressure was significantly related to LV mass including \((r = 0.26)\) (Figure 4) and excluding papillary muscle \((r = 0.27)\), but not papillary muscle mass itself. On the other hand, triglycerides \((r = −0.37)\) and HDL cholesterol \((r = −0.39)\) were solely correlated with papillary muscle mass. Total cholesterol had a direct relationship...
with LV mass ($r = 0.32$) and papillary muscle mass ($r = 0.54$) but not to LV mass excluding papillary muscle.

**Discussion**

The important finding in this study is the remarkable power of CMR to elucidate genetic variance and heritability estimates in normal humans, even with a relatively small number of twin pairs. We found heritability estimates in terms of LV mass and particularly on papillary muscle mass that are considerably greater than those reported with any other technique. To our knowledge, papillary muscle mass has not been specifically investigated previously. The finding is not trivial since the papillary muscle structure and function plays an important role in mitral valve function. For instance, an external device that repositions the papillary muscles can reduce ischaemic mitral regurgitation without compromising LV function. Finally, our imaging results show substantial correlations to systolic blood pressure and, oddly, to lipid values. Only papillary muscle mass was correlated inversely to HDL cholesterol and triglycerides. The relation between elevated blood pressure and cardiac hypertrophy is well known. Finding of a significant correlation in healthy subjects and based on a small cohort emphasizes the physiological relation as well as the measurement precision of CMR. Interindividual differences in blood pressure explain but a sub-fraction of the variance of LV mass. Even in light of the fact that our blood pressure measurement is just a snapshot and influences of blood pressure are rather integrated over time, other factors yet to be determined are relevant for cardiac mass. Since total cholesterol showed a direct relationship with both LV and papillary muscle mass, the findings are probably not spurious. We view these associations as surprising and novel in terms of their implications for normal subjects.

Our new finding is the considerable sharing of genetic variance between the LV wall and papillary muscle. Our results give evidence for an additional genetic component specific to the papillary muscle.
muscle. Thus, genetic variants may either influence both of these cardiac structures or specifically either tissue separately. Association analyses should take this issue into account by examining specific cardiac structures in subsequent genetic models.

Within-pair correlations of our CMR-based measurements are considerably higher than those reported for LVM in previous studies employing echocardiography. Even with the limitations of this relatively small sample, this finding suggests that previous studies underestimated heritability, as measurement error and inaccuracy inflate estimates of environmental influences. As the power of association studies to detect effects of genetic variants is critically dependent on measurement precision of phenotypes, CMR is superior to echocardiography and allows reduction of sample size. Despite the small sample size, we found robust partial correlations between cardiac muscle mass parameters and cardiovascular-related variables, including BMI, body surface area, dry lean mass, body fat, systolic blood pressure, and heart rate. Previously, such correlations required far larger twin studies. Cardiac magnetic resonance evidently permitted precision not available in previous studies. Expansion of this investigation should allow us to determine whether BMI, systolic blood pressure, or both are driving factors in terms of heart size.

Our study has limitations. We were only able to investigate 25 twin pairs, without DNA from parents, in this study. Thus, molecular genetic studies were not possible. However, studies such as ours that include DNA analyses from parents are of utility for linkage estimates. Our study is a pilot project. Nonetheless, we present a study that provides more robust information than earlier much larger investigations. We are encouraged that with such a small number of subjects, we could identify relatively robust results on the heritability of cardiac mass with particularly relevant information on papillary muscle. Our message is two-fold. First, CMR offers superior measurement precision and improves power regarding genetic issues. Secondly, focus on secondary structures, such as papillary muscle, may provide highly relevant clinical information.

Conflict of interest: none declared.

References

Figure 4 Relation between systolic blood pressure and left ventricular mass ($r^2 = 0.17$).