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MORPHOLOGICAL STUDIES OF THE MURINE HEART BASED ON PROBABILISTIC AND STATISTICAL ATLASES

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Abstract

MR imaging (MRI) has significant potential for quantifying cardiac function and dysfunction in the mouse. Recent advances in high-resolution cardiac MRI techniques have contributed to developing atlas-based approaches to describe anatomic structures and their function in normal and transgenic mice. This study directly compares morphological features of the mouse heart in its end-relaxed state based on constructed morphometric maps and atlases using Principal Component Analysis in C57BL/6J (n=8) and DBA (n=5) mouse strains. Comparing the constructed probabilistic atlases, a gradient probability exists for both strains in longitudinal locations from base to apex, and also shows increased variability in apical locations of both strains. Based on the statistical atlases, differences in size (49.8%), apical direction (15.6%), basal ventricular blood pool size (13.2%), and papillary muscle shape and position (17.2%) account for the most significant modes of shape variability for the left ventricle of the C57BL/6J mice. For DBA mice, differences in left ventricular size and direction (67.4%), basal size (15.7%), and position of papillary muscles (16.8%) account for significant variability. The right ventricles compared for each mouse strain yields variability in the position and size (85.1 and 68.3%), and basal and apical size and locations (12.3 and 31.6%) for C57BL/6J and DBA/2J mice, respectively.

Keywords

Magnetic Resonance Imaging; cardiac; mouse; probabilistic; statistical; atlases

Introduction

The emergence of computational anatomy [1] has traditionally been based on forming and developing complex mathematical and computational models to represent and statistically quantify anatomic structures and their morphology, using a multitude of imaging techniques. Important to the development of such models are generalized shape representations of structures, often categorized according to the methodological approach employed [2], including: (a) landmark definition and space-warping transformations, (b) data-warping transformations and subsequent use of the deformation field to a segmented object template, (c) object surface parametric modelling, (d) extraction of surface features, or (e) data extraction of the shape medical axis.

One of the most popular shape representation methods is the well known Point Distribution Model (PDM) introduced by Cootes *et al.* [3]. This method defines a mean shape model and, given the existence of a training set, computes its deformation modes using Principal Component Analysis (PCA). A direct extension of the applicability of such methods [4] is the construction of population-based atlases that rely on population statistics and encode information visually and quantitatively, based on anatomical variability. In probabilistic atlases for example, information regarding anatomical and functional variability is captured in the form of tissue probability maps—the probability of an image voxel belonging to a certain tissue structure.

Despite multiple prior efforts with construction of human brain atlases [5–9], Frangi *et al.* [10], Mitchell *et al.* [11], and Lotjonen *et al.* [12] were the first to develop human ventricular statistical shape models. More recently, Ordas *et al.* [13] developed a computational atlas of the entire heart using registration-based techniques. Perperidis *et al.* [14, 15] and Hoogendorn *et al.* [16] proposed 4D spatio-temporal human cardiac probability atlases from (MRI), while Beg *et al.* [17] developed a large deformation diffeomorphic mapping (LDDM) for construction of cardiac statistical atlases. An extension of Beg's work was recently published, where Helm *et al.* [18] employed LDMM to achieve intersubject registration to a reference anatomical template to compare cardiac geometric variability using PCA from diffusion tensor MRI in normal and failing human hearts.

Recent advances in high-field, high-resolution cardiac imaging techniques, including MRI [19, 20] and computed tomography (CT) [21] for mouse imaging, have led to the development of atlas-based approaches for the description of anatomical structures and their function [22, 23] in normal [19–21] and transgenic mice [24].

Probabilistic and statistical atlases can potentially enable studying murine global cardiac structure and function, identifying modal components of shape variability, and disseminating components of global mechanical motion. Similar to existing cardiac atlases [15, 16], such atlases can be population-based instead of single-subject atlases. These atlases can serve as a powerful reference tool for morphological and functional inter-strain mouse studies, complementary to current ongoing efforts for image-based phenotyping that target the cardiovascular system [25].

Efforts have focused on modelling cardiac anatomy in humans [18], but only limited attempts have been made to construct accurate, high-spatial and high-temporal resolution computerized atlases for mice. In particular, no atlases exist that capture the morphological variability of mouse hearts across different strains and types, as well as under inhalational anesthesia, a prerequisite for non-invasive imaging studies.

This study presents constructions of computational probabilistic and statistical atlases from cardiac MRI as a platform for controlled inter-strain, anatomical phenotypic analysis across two different normal mouse strains (C57BL/6J and DBA/2J) in their end-relaxed state.

The morphometric map constructions are constrained by using Point Distribution Models (PDM) as the shape representation method using Principal Component Analysis (PCA). In this work, a segmented (template) reference MRI sequence is selected from each of the two mouse databases consisting of imaged anatomic structures (left and right ventricular myocardium, left and right ventricular blood pools, and papillary muscles) as representative sets of typical anatomic objects of interest for C57BL/6J and DBA/2J mice. This template is subsequently used to register population images via global and local non-rigid transformations. Although such high-dimensional transformation differs from established one-to-one (invertible) diffeomorphic transformations [18], its performance is comparable in accuracy, precision, simplicity, and computational intensity, as recently reported by a multi-

center quantitative ranking of 14 different non-linear deformation algorithms [26]. Shape variations are subsequently assessed using PCA and primary modal components of shape variation are determined. This work establishes the platform for further work to compare human morphological and functional studies, for statistical inference on cardiac function and dysfunction from mouse to human.

Methods

Mouse Physiology and Animal Preparation

All animal studies were approved by the Duke University Institutional Animal Care and Use Committee. A total of 8 male C57BL/6J (mean weight $\pm$ sd, 25.3 $\pm$ 3.0g) and a total of 5 male DBA (26.2 $\pm$ 6.3g) mice were used. Animals were anesthetized with 1–2% isoflurane through a nose cone. All animals were injected in a single bolus via the tail vein with a liposomal contrast agent at a dose of 0.2mmol/kg. The contrast agent, described previously [27], increased myocardial tissue contrast of left ventricular blood cavity and muscle. The contrast agent consisted of liposomes (~100nm in diameter) filled with GdDTPA (Magnevist, Bracco Diagnostics, Princeton, NJ). The surface of the liposome is coated with polyethylene glycol resulting in a blood half-life >1 hour, more than sufficient for the ~45-minute acquisition.

MRI Methodology

Hardware – Pulse Sequence – Reconstruction—All imaging was performed at the Duke Center for In Vivo Microscopy (CIVM), an NCRR national Biomedical Technology Research Center. Images were acquired on a 7T Magnex magnet interfaced to a GE EXCITE console (Epic 12.4, GE Medical Systems, Milwaukee, WI) modified for small animal imaging.

Briefly, the imaging protocol described previously [20], employs a 3D radial encoding technique, which captures signal from the free induction decay following a volume excitation. This allows acquisition of data with very short “effective” echo times (TE <200 μ sec) where the first data point is captured 150–200 μ sec after the end of the excitation pulse. Gradients are cycled on all three axes to define radial trajectories in the Fourier volume. The simplicity and compactness of the sequence permitted encoding at very short repetition times (TR <3.1ms). Data are prospectively gated on the peak R-wave. The R-R interval, ranging from 110–130ms, is divided prospectively into 8–10 time frames. After the initial trigger on the rising part of the R wave, the sequence sequentially fills 8–10 different raw data volumes, each representing a time span of approximately 10–15ms in the R-R interval. Four-to-six views are acquired for each of the individual sub-volumes per cardiac cycle. During subsequent cardiac cycles, additional views are acquired (4–6 views/time point) for each temporal sub-volume. In total, 62,720 views are acquired for each sub-volume, which represents an under-sampling factor of ~3 for the entire selected 3D volume. Data are re-gridded onto a 256x256x256 array with effective isotropic resolution of 110x110x110 μ m³ (voxel volume=1.3nl). One C57BL/6J dataset was re-gridded to isotropic resolution of 87x87x87 μ m³ and one DBA dataset to non-isotropic resolution of 110x110x348 μ m³. Such sets were interpolated using tricubic/spline interpolation to the target resolution of 110x110x110 μ m³. The C57BL/6J set was subsequently used as the reference template set for the co-registration of the remaining sets in the C57BL/6J cohort. The total acquisition time for an 8x256³ array was ~45 minutes. Image SNR ranged between 13–42.7 for left ventricular blood and 4.8–16.4 for left ventricular myocardium for C57BL/6J mice and 9.1–46.7 and 3–15.4 for corresponding regions of DBA mice. A base-to-apex myocardial signal fall-off was recorded within the two cohorts.

Image Processing

Atlas Construction: To construct a 3D mouse atlas, the initial steps for both the probabilistic and statistical atlases are identical:

- **Step 1:** Segment the end-diastolic image sequences (spanning the heart from base to apex) into left ventricular, right ventricular blood cavities, and myocardium using manual seed-point segmentation
- **Step 2:** Volume render and register the images sequences to a common coordinate system to enable direct comparison of cardiac anatomies using specified landmarks
- **Step 3:** Blur the transformed segmented image sequences with a three-dimensional (3D) Gaussian filter with a standard deviation of $\sigma=2$ voxels to account for uncertainties in segmented tissue borders

The probabilistic atlas can then be constructed by averaging the transformed segmented and volume-rendered images of the right ventricle and left ventricular myocardium. The result is a map of probabilities of these two structures.

Construction of the statistical atlas requires the following additional steps:

- **Step 4:** Extract the surfaces from each volume rendered segmented image sequence and generate pseudo-landmarks using the *marching cubes* algorithm [28]
- **Step 5:** Automatically propagate a set of pseudo-landmarks from a landmarked template (reference) model to the rest of the surface models in each cohort by establishing direct correspondence between homologous points using a non-rigid registration method
- **Step 6:** Perform a statistical analysis in the form of Principal Component Analysis (PCA) on the position of the propagated pseudo-landmarks

The following paragraphs describe in detail the procedural steps 1–6:

Segmentation (Step 1): Left and right ventricular blood cavities and myocardium were manually segmented by an expert user (Analyze 7.0, Mayo Clinic, USA) from end-diastolic cardiac MRI phases. Shortaxis images (50–60 total per cardiac phase) with isotropic spatial resolution covering the entire heart and large vessels were analyzed. Seed-point spline contours were defined to delineate the borders of the endocardial, epicardial, and right ventricular surfaces. The thin right ventricular (RV) myocardial wall and the low image quality defining high-frequency features of the RV wall necessitated definition of its boundary as the endocardial RV blood cavity. When binary masks were generated, left ventricular myocardial, left ventricular cavity, and right ventricular masks were intensity normalized and converted to the .gipl format (Guy's Hospital, UK) using ImageJ (ImageJ, NIH, Bethesda, USA). Uncertainties in mask construction in apical regions of the mouse hearts were corrected by a different expert user. using the RView program (IXICO, UK) Masks were then converted to 3D volume stacks and saved in standard Analyze format.

Registration (Step 2)

Global Registration: To enable direct comparison of the cardiac anatomies of mice in the same cohort, such anatomies were first mapped on the same image coordinate reference system. It is critical that the mapping transformed all such mouse anatomies to the same coordinate system to maintain their local shape characteristics. Before producing the probabilistic atlas, each segmented image sequence (stack) is blurred with a 3D Gaussian kernel with $\sigma=2$ voxels and the affine transformation is applied (Step 3). Such *a-priori*

operation addressed this problem by modelling this uncertainty in tissue classification. Then, a simple affine registration method was used to align all mouse hearts.

The use of transformation mapping related each spatial point in a particular mouse image surface to the corresponding reference surface according to:

$$\mathbf{T}(x, y, z) = \mathbf{T}^{global}(x, y, z) \forall O_k \quad (1)$$

where x, y, z represent image and slice spatial locations for all k mouse species O_k . $\mathbf{T}^{global}$ was an affine transformation with 12 degrees of freedom that captures differences in the size, orientation, and alignment of the hearts, according to:

$$\mathbf{T}^{global}(x, y, z) = \begin{pmatrix} \theta_{11} & \theta_{12} & \theta_{13} \\ \theta_{21} & \theta_{22} & \theta_{23} \\ \theta_{31} & \theta_{32} & \theta_{33} \end{pmatrix} \times \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} \theta_{14} \\ \theta_{24} \\ \theta_{34} \end{pmatrix} \quad (2)$$

The coefficients of Equation [2] parametrize the 12 degrees of freedom of the transformation. Given the limited number of degrees of freedom of the global transformation, the registration was based on the definition of 3 landmarks for RV and 5 landmarks for LV anatomy. These landmarks were identified by an expert user in each separate image of all the image sequence (stacks), before constructing the surface renditions. They were subsequently placed on the RV and LV surfaces according to the radial method described by Beg *et al.* [17]. The affine registration used IRTK software (IXICO Ltd.) [29, 30].

Local Registration and Pseudo-Landmark Propagation (Steps 4 and 5): A representative (template) image sequence was used to generate a reference surface rendition. Extracted surfaces from all other image stacks (from all remaining mice in the same cohort) are registered to the reference surface using the non-rigid global registration method. Post-completion of global registration, a local transformation was applied to propagate the pseudo-landmarks of the reference surface to the surfaces of the remaining mouse cohorts.

The marching cubes algorithm [28] was used to generate a dense triangulation of the boundary surface (Step 4) of each anatomical structure (left, right ventricular blood cavities, and myocardium). This methodology has been used previously by Frangi *et al.* [31] for the construction of human cardiac atlases. The vertices of the triangulation mesh serve as pseudo-landmarks. To perform statistical analyses on mouse datasets, correspondence between the pseudo-landmarks of constructed end-diastolic endocardial and epicardial surfaces is first established. To achieve pseudo-landmark correspondence, a non-linear surface registration based on B-spline models is employed [32] (part of the IRTK software).

In this case, the transformation is a composed operation defined by:

$$\mathbf{T}(x, y, z) = \mathbf{T}^{local} [\mathbf{T}^{global}(x, y, z)] \quad (3)$$

where $\mathbf{T}^{global}$ defines the global transformation mapping that aligns the images in the same coordinate system correcting for global shape differences (as described in the previous section), and $\mathbf{T}^{local}$ represents a transformation mapping accounting for local non-linear shape differences (thereby establishing correspondence between the pseudo-landmarks).

The local transformation mapping, $\mathbf{T}^{local}$, is expressed as a 3D product of the familiar onedimensional (1D) cubic B-splines [31] defined by:

$$\mathbf{T}^{local}(x, y, z) = \sum_{l=0}^3 \sum_{m=0}^3 \sum_{n=0}^3 B_l(u) B_m(v) B_n(\omega) \phi_{i+l, j+m, k+n} \quad (4)$$

where $n_x \times n_y \times n_z$ denotes a mesh of control points ϕ along the Cartesian coordinate axes,

$i = \left\lfloor \frac{x}{n_x} \right\rfloor - 1, j = \left\lfloor \frac{y}{n_y} \right\rfloor - 1, k = \left\lfloor \frac{z}{n_z} \right\rfloor - 1, u = \frac{x}{n_x} - \left\lfloor \frac{x}{n_x} \right\rfloor, v = \frac{y}{n_y} - \left\lfloor \frac{y}{n_y} \right\rfloor, \omega = \frac{z}{n_z} - \left\lfloor \frac{z}{n_z} \right\rfloor$, $[\cdot]$ represents the floor function, and B_l represents the l^{th} basis function of the B-spline.

Probabilistic Map: A 3D probabilistic atlas depicts the spatial probability $p_s(x, y, z)$ that a structure s appears at location x . In the probabilistic atlases, small voxel intensity value reflects smaller probability that a particular voxel belongs to a particular structure (a voxel intensity of 255 corresponds to a probability of 1, while an intensity of 0 corresponds to a probability of 0).

A common problem in constructing a probabilistic atlas arises where mouse image sequences do not exactly match spatially, for example where spatial mismatches exist in the locations of apical and basal slices in separate mice studied with the same acquisition protocol. To ensure consistency in probability calculations, the averaging operation is performed only for matching spatial images (slices).

The probabilistic atlas $\mathbf{A}(x, y, z)$ has the same voxel dimension as the reference image used for registration. Such an atlas, $\mathbf{A}$, is formed by averaging the corresponding transformed, blurred, segmented, images of the O_k mouse species used to construct the atlas $\{O_k; k=0 \dots n_p\}$ according to:

$$\mathbf{A}(x, y, z) = \frac{1}{n_p} \sum_{k=0}^{n_p} O_k(x, y, z), \quad \forall O_k \quad (5)$$

where n_p is the total number of mice in each cohort, x, y are in-plane and z is the out-of-plane (slice) spatial location for each mouse O_k used to construct the atlas.

Statistical Map (Step 6): Let $\{q_i; i=0 \dots n_p\}$ denote the subspace of n shapes from p species (n_p). Each shape consists of m 3D landmarks, $\mathbf{P}_j = (p_{1j}, p_{2j}, p_{3j}; j=1 \dots m)$. The aim of the statistical analysis is to approximate the distribution of the landmarks with a linear model of the form:

$$\mathbf{q} = \bar{\mathbf{q}} + \Phi \mathbf{b} \quad (6)$$

where $\bar{\mathbf{q}}$ is the average landmark vector, $\mathbf{b}$ is the shape parameter vector of the model, and Φ is a matrix of eigenvectors. The matrix Φ is obtained by performing PCA on the covariance matrix $\mathbf{C}$ [33]. Using PCA, the principal components of $\mathbf{C}$ are calculated as its eigenvectors ϕ_j and corresponding eigenvalues λ_j (in ascending order such that $\lambda_i < \lambda_{i+1}$).

New shape examples can be generated by varying the shape parameter vector $\mathbf{b}$ ($b_{within}, b_{between}$) [Eq. 6] as previously demonstrated [3]. Assuming that the data distribution is described by a multi-dimensional Gaussian distribution, the variance of the i^{th} parameter of $\mathbf{b}$ across the training set is given by λ_i . If limits are imposed in the variation of b_i such that $b_i \leq \pm 3 \sqrt{\lambda_i}$ then the generated shape is similar to that contained in the training class.

Landmark Registration and Propagation Error

Global Registration Error: The computational error of surface landmark placement was calculated for various landmark points (N_l), as a root-mean-squares error cost function, $\bar{\epsilon}_g$, of landmark positions according to:

$$\bar{\epsilon}_g = \frac{1}{N_l} \sqrt{\sum_i^{N_l} \left[\left\| \ell_{O_{reference_i}}(x, y, z) \right\| - \left\| \ell_{O_{k_i}}(x, y, z) \right\| \right]^2} \quad (7)$$

and as the absolute mean Euclidean distance error $\bar{\epsilon}_{d,g}$:

$$\bar{\epsilon}_{d,g} = \frac{1}{N_l} \left| \sum_i^{N_l} \left[\left\| \ell_{O_{reference_i}}(x, y, z) \right\| - \left\| \ell_{O_{k_i}}(x, y, z) \right\| \right] \right| \quad (8)$$

where N_l is the number of total landmark points placed by the user, and $\|\ell_{O_{reference_i}}\|, \|\ell_{O_{k_i}}\|$ are the vector norms of the landmark locations of the reference and k mouse species $O_{reference}$ and O_k , respectively.

Local Registration Error: To quantify the error associated with the propagation of pseudo-landmarks on the reference (template) surface (generated by the marching cubes algorithm) to each of the surfaces of mice in each cohort, a root-mean-squared error cost function $\bar{\epsilon}_l$ was defined in a similar fashion to Eq. [7] above as:

$$\bar{\epsilon}_l = \frac{1}{N_{pl}} \sqrt{\sum_i^{N_{pl}} \left[\ell_{O_{reference_i}}(x, y, z) - \ell_{O_{k_i}}(x, y, z) \right]^2} \quad (9)$$

where N_{pl} is the total number of pseudo-landmark points generated. Any error associated with the direct correspondence of pseudo-landmarks (in addition to segmentation errors) will propagate in the PCA analysis.

Statistical Analysis: All results are reported as mean (and whenever appropriate mean $\pm$ standard deviation [SD]). Two-way unbalanced analysis of variance (ANOVA) tests were also used (XLSTAT, Addinsoft, USA) to disseminate significant variability effects of mean probability interactions between strain, slice, and area locations from base to apex in both mouse cohorts. Two-tailed unpaired student t-tests were employed to assess statistical significance in mean probabilities of corresponding segmented short axis regions (anterior, lateral, inferior, and septal) for the two constructed probability atlases for C57BL/6J and DBA/2J mouse strains using StatPlus (AnalystSoft, UK) at the 1% significance.

Results

Table 1 lists physiological and image acquisition details for the C57BL/6J and DBA/2J mouse cohorts. Figure 1 depicts typical true short and long axis cardiac images using the view order randomization achieved by the pulse sequence employed for MRI, as described previously [20]. Also shown are results for seed-point segmentation, binary mask formation, and subsequent RView boundary corrections.

Figure 2a depicts a graphical illustration of the automated landmarking process used for local registration. The landmarks are propagated from one particular case per species, using non-rigid surface-based registration. Landmark propagation RMS errors were marginal and ranged between 0.11–0.35 voxels for LV and between 0.02–0.11 voxels for the RV of

C57BL/6J mice, and between 0.07–0.34 and 0.05–0.07 voxels for LV and RV, respectively, for DBA mice, indicative of accurate local registration. Figure 2b shows the entire process, including global and local registration. The generated binary masks aid in the construction of the 3D model surface rendering and tetrahedral finite element mesh construction.

Table 2 lists mean root-mean-squared (RMSE) and mean Euclidean distance errors for global registration for various landmarks (N=3–9) for the RV and LV surfaces. For C57BL/6J mice, the RMSE and absolute Euclidean distance errors for five landmarks defined on the LV epicardial surface are 1.30 and 0.86 voxels, respectively, indicative of the accuracy of the registration process. The corresponding RMSE and distance errors for mice of strain DBA were 1.91 and 2.12 voxels, respectively. For global registration of the RV, three landmark points were used that resulted in mean RMSE and absolute Euclidean errors of 1.76 and 1.59 voxels for C57BL/6J, and 3.25 and 1.93 voxels for DBA mice, respectively.

Probabilistic Atlas

Figure 3 shows the probabilistic atlases of C57BL/6J mice, with Figure 3a showing the short axis and long axis views of the myocardial atlas. Figure 3b depicts the corresponding right ventricular atlas of the same strain. Figures 3c and 3d show the atlases of the myocardium and right ventricle displayed on the same spatial coordinate system for DBA mice. Figure 4 is a graphical illustration of the slice and area locations (Anterior, Lateral, Septal, Inferior) of short axis (Basal, Mid, Apical) slices of the constructed probability maps used for quantitative comparisons. Regions of interest (ROIs) were selected in different longitudinal slices of the two strains for comparison. Mismatches in the selected longitudinal slice locations (due to possible interspecies cardiac size variations) were accounted for, by normalizing the total ROI area pre-selected in C57BL/6J mice with the ratio of the circumferential area (CA) to the base-apex (long-axis) distance (BA_d), according to:

$$ROI_{DBA} = ROI_{C57BL} \cdot \frac{\left(\frac{CA}{BA_d}\right)_{C57BL}}{\left(\frac{CA}{BA_d}\right)_{DBA}} \quad (10)$$

Selected ROI areas ranged between 715–1006 and 643–922 square voxels for basal slices for the C57BL/6J and DBA strains, between 730–793 and 608–909 for mid-slices, and between 229–398 and 114–471 square voxels for apical slices for the same mice in the two cohorts.

Table 3 lists the mean probabilities in the selected areas and slices of the two mouse cohorts and confirms a base-apex probability gradient in mean probabilities, with apical locations exhibiting lower mean probabilities in comparison to basal and mid-slices. Furthermore, significant differences were noted in corresponding basal-lateral, mid-anterior, and apical-septal locations of the two mouse cohorts studied.

Two-way ANOVA analysis indicated low variability in mean myocardial probability as explained by the model parameters of myocardial area, slice location, mouse strain, and their interactions as reflected by goodness of fit and estimated low coefficient of determination $R^2 = 0.105$. For such constructed model, a significant effect was noted due to the three-dependent variables and their interactions was noted ($p < 0.001$) with in-slice myocardial areas exhibiting the highest impact on myocardial probability variation. Noted also was a significant effect on mean probability from apical locations in comparison to basal and mid-slice locations.

Statistical Atlas

The statistical atlas of the left ventricular myocardium C57BL/6J mice contains 16160 points for the left ventricle and 7388 points for the right ventricle. The corresponding atlas for DBA mice contains 19572 left ventricular myocardial points and 10114 right ventricular points. Figure 5 shows model renditions of the three most significant variation modes for the myocardium for both mouse strains, while Figure 6 shows the corresponding modes of variation of the right ventricle for mice with the same strains.

For the C57BL/6J left ventricular myocardium, the most significant shape changes that account for up to 95% of shape variation as described (Table 4) by difference in size (explaining 49.8% of total shape variation); the remaining modes of shape variation describe apical direction (15.6%), the size of ventricular pools and direction of left ventricular myocardium (13.2%), papillary muscle shape and location (16.2%) and higher orders of variational difference. Similar to left ventricular myocardium, the most significant shape variation modes for the right ventricle (Table 4), describe position and size of the RV (85.1%), basal position (10%), apical and basal size (5.0%).

Correspondingly, for DBA mice (Table 4), size and direction of left ventricular muscle explains 67.4% of shape variation, while basal myocardial size (15.7%) and position of papillary muscles (16.8%) explain the remaining modes of highest variability in shape. For the RV, variability is explained by the size of the right ventricle (68.3%), basal and apical size, and ventricular direction (18.1%), and its basal size-apical location (13.5%).

Discussion

This study summarizes comparative statistics of inter-strain cardiac morphology in mice. Ultimately, the accuracy of constructed probabilistic and statistical atlases depends on accuracy of the segmentation, registration, and landmark propagation procedures. These quantitative analyses were based on segmentation by expert user seed-point segmentation followed by additional RView correction.

The employed registration method is based on the use of a linear transformation model. Such a linear model aligns the cardiac anatomic changes or changes to their local shape characteristics. Registration analyses indicated accurate global registration as reflected by mean Euclidean landmark distance error of value <2 voxels and as justified by a recent multi-center inter-comparison of 14 different registration methods, including the IRTK registration used in this work [26]. According to the quantitative results of Table 1, the error associated with increasing number of seed points is not linearly increasing. Furthermore, the registration error will not necessarily decrease as the number of seed-registration points increases. Increasing the number of selected registration points in our analysis did not result in better alignments of the mouse species. In this work, the identification and placement of landmark points was based on prior published reports on cardiac registration using the radial method described by Beg *et al.* [17]. Other seed-point registration placement techniques were also identified in the referred publication, however, the unique anatomy of the heart does not permit easy and reproducible placement of hundreds of anatomic landmarks. Use of numerous anatomic landmarks is expected to exacerbate non-linear differences during the registration process. Additionally, selection of a different transformation model is not preferred, since the affine model does not affect the cardiac anatomy locally. Affine transformation models are the established models for the construction of probability atlases and have been used extensively by a number of investigators [31, 33–35]. Methods that utilize non-rigid registration algorithms have also been presented previously for the construction of statistical deformation field atlases [9]. However, the goal of this study was the alignment of the mouse hearts without altering or affecting local shape characteristics.

Constructed probabilistic and statistical atlases contain information regarding shape variability of the data. The probabilistic atlases capture information about cardiac anatomy using tissue probability maps. Despite their value, probability maps provide somewhat limited information regarding the nature of variability in shape and function of anatomical structures—a limitation that is ameliorated by the statistical atlases. Contrary to probabilistic atlases, statistical atlases provide, in addition to inherent data variability that may exist, the exact type of such variability.

Comparing the myocardial probabilistic atlases of the two strains studied, a longitudinal gradient of mean probability exists for both strains in basal-to-apical locations. These data reason in favor existing variability in the apical location in both strains, a direct consequence of the heart's effort to re-establish the position of the apex in a consistent manner at end-diastole. Additionally, higher variability exists in DBA mice in the location of the papillary muscles, as well as in the epicardial areas of the left ventricle.

The statistical analysis was performed separately for each anatomical structure. Performing a statistical analysis on all structures of the entire heart may capture and unmask interesting variations in shape. Based on the constructed statistical atlases, differences in size, apical direction, papillary muscle shape and position, and basal LV blood cavity size, account for the most significant modes of shape variability. Similarly, comparison of the right ventricles of the two strains yielded variability accounted for by size, position, and direction of the RV, especially at apical locations.

It is expected that in future studies, increasing the mouse population will result in increased quantitative accuracy of constructed models (both statistical and probabilistic), establishing them as more representative of the parent-population.

Although the quantitative accuracy of established MRI cardiac functional techniques (including DENSE, spin-tagging, and others) supersedes the accuracy of the probabilistic and statistical models constructed in this study, such an inter-strain comparative attempt avoids complicated development and post-processing analyses, often associated with such imaging methods.

Future work will complement such analyses to the entire four-dimensional cardiac cycle (systolic and diastolic phases) disseminating functional effects due to the motional components during the entire cardiac cycle. Improvements in the current model will include additional strains, and inclusion of the atria. Additional value to such analyses is envisioned through model-based global fitting of the endocardial and epicardial surfaces facilitating quantitative estimation of cardiac mechanical indices and stress-strain comparisons.

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List of Abbreviations

PCA Principal Component Analysis

PDM	Point Distribution Model
LDDM	large deformation diffeomorphic mapping
CT	computed tomography
CIVM	Center for In Vivo Microscopy
LV	left ventricle
RV	right ventricle
1D	one-dimensional
3D	three-dimensional

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Figure 1. Basal diastolic short and long axis MR images of a (top row) C57BL/6J and a (second row) DBA/2J mouse study. (Third row) Seed-point segmentation and binary mask construction in short axis MRI; (fourth and fifth rows) RView 3D binary mask correction.

Figure 2.

(A) Graphical representation of landmarking. The landmarks are propagated from a reference landmarked template heart model to each remaining mouse heart of each cohort. (B) Typical mid-short and long-axis images from a mouse heart; (C) RVView corrected binary masks; (D) Example of five-point landmarked mesh for global registration; (E) 3D epicardial/endocardial reference surface model rendition used for local registration, and (F) construction of the three-dimensional (3D) mesh volumetric dataset using the marching cubes algorithm.

Figure 3.

[Top] Probability atlas for mice with strain C57BL/6J. (A) Short and long axis views of the probabilistic atlas of the left ventricular myocardium; (B) corresponding views of the right ventricle. [Bottom] Probability atlas for mice with strain DBA/2J. (C) Short axis and long axis views of the probabilistic atlas of the left ventricular myocardium; (D) corresponding views of the right ventricle.

Figure 4.

[Top row] Locations of the selected basal, mid, and apical short axis slices from the constructed probabilistic atlases of C57BL/6J and DBA/2J mouse strains for quantitative probability analyses. [Mid and bottom rows] Sectorial representations of anterior (A), septal (S), inferior (I), and lateral (L) left ventricular regions for C57BL/6J mice.

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Figure 5.

Three-dimensional model renditions depicting the three most significant modes of shape deviation of the statistical atlas of the left ventricular myocardium for mice with (top) C57BL/6J and (bottom) DBA/2J strains.

Figure 6. Three-dimensional model renditions depicting the three most significant modes of shape deviation of the statistical atlas of the right ventricle for mice with (top) C57BL/6J and (bottom) DBA/2J strains.

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Table 1
Physiological and image data acquisition details for the C57BL/6J and DBA/2J mouse cohorts.

STRAIN	AGE	SEX	WEIGHT (g)	NUMBER (n)	PROTOCOL	ARRAY SIZE	SPATIAL RESOLUTION (μm^3)	TEMPORAL RESOLUTION (ms)
C57BL/6J	8–12.5 weeks	Male	25.0 $\pm$ 3.0	8	High spatial resolution	256 $\times$ 256 $\times$ 256	110 $\times$ 110 $\times$ 110 (n=7) and 87 $\times$ 87 $\times$ 87 (n=1)	2.4–3.1
DBA	8–12 weeks	Male	26.2 $\pm$ 6.3	5	High spatial resolution	256 $\times$ 256 $\times$ 256	110 $\times$ 110 $\times$ 110 (n=4) 110 $\times$ 110 $\times$ 348 (n=1)	2.4

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Table 2

Average Root-Mean-Squared (RMSE) and absolute Euclidean distance registration errors (in voxels) for various landmarks points placed on the left and right ventricles by an expert user for C57BL/6J and DBA/2J mice.

		N=3	N=5	N=6	N=7	N=8	N=9
MEAN RMSE (EUCLIDEAN DISTANCE) LANDMARK ERRORS							
C57BL/6J	LV [n=8]	-	1.30 (0.86)	1.96 (0.89)	1.99 (0.35)	1.85 (0.93)	1.90 (0.18)
	RV [n=8]	1.76 (1.59)	1.34 (0.04)	2.06 (2.34)	2.47 (2.85)	2.82 (3.13)	2.83 (4.00)
DBA/2J	LV [n=5]	-	1.91 (2.12)	2.50 (2.02)	2.86 (2.32)	2.97 (1.56)	2.97 (2.06)
	RV [n=4]	3.25 (1.93)	1.09 (1.78)	2.38 (1.93)	2.86 (0.06)	2.72 (0.46)	3.46 (3.40)

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Table 3

Mean probability measurements from probabilistic atlases in various left ventricular regions (anterior, septal, inferior, lateral) within short axis slices of C57BL/6J and DBA/2J mouse strains.

MEAN PROBABILITY (Pr)	BASE				MID				APICAL			
	Anterior	Septal	Inferior	Lateral	Anterior	Septal	Inferior	Lateral	Anterior	Septal	Inferior	Lateral
C57BL/6J	0.38	0.36	0.38	0.36 [‡]	0.38 [‡]	0.34	0.36	0.30	0.13 [‡]	0.14 [‡]	0.13	0.14
DBA/2J	0.39	0.36	0.39	0.34	0.42	0.31	0.35	0.29	0.13	0.09	0.11	0.12

[‡] Statistically significant relative to the corresponding region at the same slice location of the mouse population with a different strain at the 1% significance level.

Table 4

Tabulated comparison of the most significant modes of shape variation of the statistical atlas of the left and right ventricular myocardium for C57BL/6J and DBA/2J mouse strains using Principle Component Analysis.

STATISTICAL ATLAS	STRAIN	MODE	PERCENTAGE OF SHAPE VARIATION	DESCRIPTION OF VARIATION
LV	C57BL/6J	1	49.8	Size of myocardium
		2	15.6	Apical direction
		3	13.2	Size of the base of ventricular blood pools and direction of left ventricular myocardium
		4	9.3	Papillary muscle shape and position
		5	6.9	Papillary muscle shape and position
		6	3.0	Higher order of variational difference
		7	2.1	Higher order of variational difference
	DBA	1	67.4	Size and direction of myocardium
		2	15.7	Size of the base of heart
		3	11.8	Position of papillary muscles
		4	5.0	Position of papillary muscles
RV	C57BL/6J	1	42.4	Position and size of right ventricle
		2	23.1	Position of right ventricle
		3	19.6	Position of right ventricle
		4	10.0	Basal position
		5	2.3	Apical and basal size
		6	1.6	Size of right ventricle
		7	1.1	Size of right ventricle
	DBA/2J	1	68.3	Size of right ventricle
		2	18.1	Basal size and apical size and direction of right ventricle
		3	13.5	Size of right ventricle's base and apical location