

Structural Differences in Transgenic Animals Associated with Atrial Fibrillation

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Abstract

The mechanisms that drive and sustain atrial fibrillation (AF) continue to be a highly researched topic. Atrial fibrosis has been linked with increased incidence of AF and conduction, but how fibrosis may lead to AF sustaining remains unknown. Our study aims to highlight heterogeneity in atrial fibrosis and how differences in fibrotic architecture may influence the sustainability of AF. In our study, we utilize a transgenic goat model with cardiac-specific over-expression of TGF β -1 gene to examine structural differences of the fibrotic regions between animals that are inducible for AF and animals that remain AF-free. Our results indicate that there are structural differences between the fibrotic regions of AF inducible and non-inducible animals. Animals inducible for AF were found to have increased structural isotropy and increased fiber disarray within the fibrotic regions. Histology samples taken from the fibrotic regions showed fibrotic strands disrupted the tissue fibers in a more obstructive manner in the inducible animal group. These results highlight the heterogeneous differences of fibrotic regions.

1. Introduction

Atrial fibrillation is the most common cardiac arrhythmia worldwide, and it has been associated with electrical, structural, and contractile remodeling.[1] Previous studies have associated structural remodeling with a higher incidence of AF,[2] and regions of structural remodeling have been associated with conduction slowing.[3] Additionally, fibrotic differences have been reported as AF is induced and sustained in animals.

Using a transgenic animal, we evaluated structural dif-

ferences within the fibrotic regions between animals that are inducible for AF and those that remain AF-free. With a transgenic model, we independently examine the role of structural remodeling without added complicating factors such as electrical remodeling. Previous studies have suggested a difference in fiber orientation between inducible and non-inducible transgenic goats.[4] Our study will examine structural differences such as structural anisotropy, fiber direction, and fibrotic architecture.

2. Methods

All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals,[5] with a protocol approved by the Institutional Animal Care and Use Committee at the University of Utah.

2.1. Animal Model:

A transgenic goat model ($n = 12$) with cardiac-specific over-expression of TGF- β 1 was used for this study.[6] The large-animal transgenic model provides a unique opportunity to study the effects of structural remodeling on sustaining AF. The model has a natural increase in structural remodeling without introducing electrical remodeling.

To test whether the transgenic animals were inducible for AF, a rapid pacing protocol was used. animals were anesthetized with propofol ($5 - 8\text{mg/kg}$) and then intubated for general anesthesia with isoflurane ($1.5 - 4\%$). A catheter was placed in the right atrium to test for AF inducibility. Pacing at 50Hz was performed at $2\times$ the diastolic pacing threshold. Animals sustaining AF for greater than 20mins without interruption were labeled as inducible for AF.

2.2. MRI:

Hearts were excised for ex-vivo MRI. A 7T scanner (Bruker) was used to perform a T1-weighted scan ($195\mu\text{m} \times 195\mu\text{m} \times 195\mu\text{m}$) and a diffusion tensor imaging (DTI-MRI) scan with 12 sampling directions with a voxel volume of 0.96mm^3 . The high-intensity regions of the T1-weighted scan indicate increased extracellular space/fibrosis. The MRI was imported into Seg3D (University of Utah), and the LA wall was segmented. Using a dynamic threshold of the myocardial image intensity, the LA was separated into high and low-intensity regions.[7] The DTI-MRI scan and the LA wall segmentation from the T1-weighted scan were imported onto DSistudio to calculate the fractional anisotropy (FA).[8] FA measures the structural anisotropy and was reported as a ratio. An FA score of 1 indicates complete anisotropy, while an FA score of 0 indicates complete isotropy. The fiber direction throughout the atria is obtained by determining the first eigenvalue of the tensor matrix. Fiber disarray was measured using a fiber direction heterogeneity score. Fiber direction heterogeneity was calculated by determining the angle difference between the fiber direction at each point to the fiber direction of its neighbors. A greater fiber direction heterogeneity score indicates increased fiber disarray.

2.3. Histology:

Tissue samples were taken from both the labeled healthy and fibrotic regions of the two groups. Samples were fixed and embedded in paraffin for Masson's Trichrome staining. Tissue sections $500\mu\text{m}$ below the endocardial surface were stained to visualize the fibrosis. The fibrotic percentage of each region was calculated using a custom-built Matlab script. The fibrotic and healthy tissue strands were identified and labeled by automatically segmenting and labeling each section. A connected components method was used to label individual fibrotic strands. Fibrotic strands greater than $100\mu\text{m}$ long were classified as obstructive strands. The number of obstructive strands per mm section was counted. For each histology sample, the general tissue fiber direction was determined. The orientation angle of each obstructive fibrotic strand in the sample was then compared to the general tissue direction. The angle difference between the fibrotic strands and tissue fiber direction was determined.

2.4. Data Analysis:

Comparisons between animals were reported as mean $\pm$ standard deviation. Structural differences between the fibrotic regions of the inducible and non-inducible animals were compared and were reported as mean $\pm$ standard error. $P < 0.01$ was considered statistically significant.

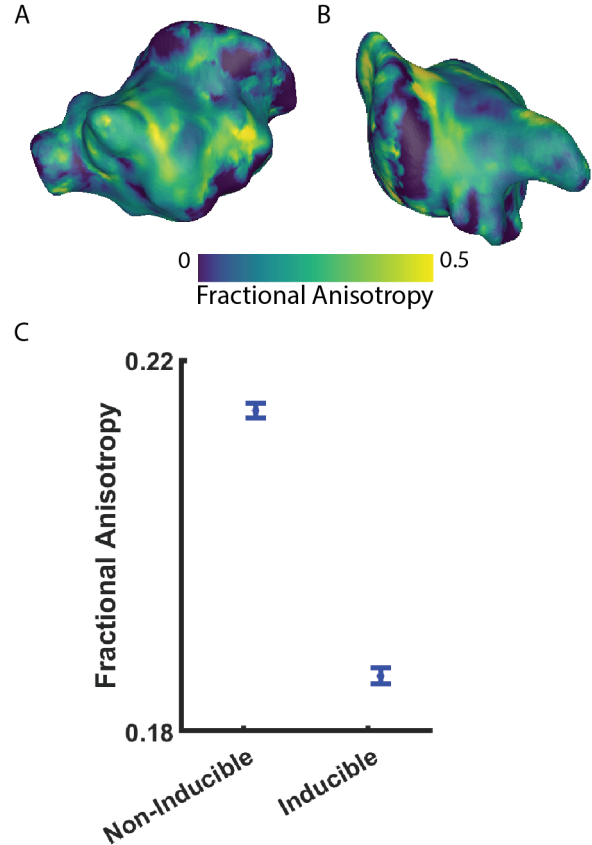


Figure 1. Fractional anisotropy differences between fibrotic regions of the inducible and non-inducible animals. A) The FA mapped onto the 3D geometry in a non-inducible animal. B) The FA mapped onto the 3D geometry of an inducible animal. C) There is lower FA in the fibrotic region of the inducible animals compared to the fibrotic regions of the non-inducible animals.

3. Results

In total, 12 animals were used for this study. 6/12(50%) of the animals were inducible for AF. The overall fibrosis found in all 12 animals was $34.2 \pm 14.3\%$. There was no statistical significance between overall fibrosis amount in the LA between inducible and non-inducible animals ($39.6 \pm 12.9\%$ in inducible animals vs $28.7 \pm 14.5\%$ in non-inducible animals, $p = 0.197$).

From the DTI-MRI, structural differences between the fibrotic region of the inducible and non-inducible group was observed. The inducible animals had a lower FA compared to the non-inducible animals (0.186 ± 0.001 vs 0.215 ± 0.001 , $p < 0.001$). This is illustrated in Figure 1. The fiber distribution between the fibrotic regions of the inducible and non-inducible animals was found to be statistically significant. There was increased fiber disarray as measured by an increase in fiber direction heterogeneity score in the fibrotic regions of the inducible animals compared to the non-inducible animals ($35.7 \pm 0.2\%$ vs

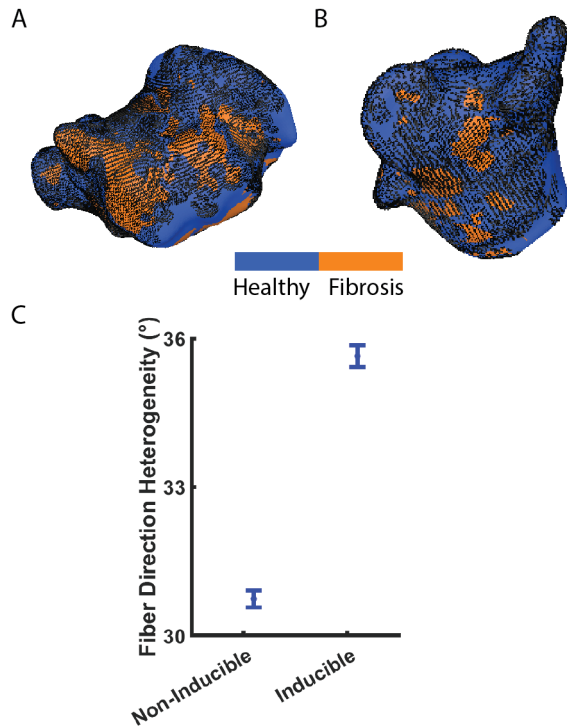


Figure 2. Fiber disarray is more prevalent in the fibrotic regions of the inducible animals compared to the non-inducible animals. Fiber directions overlaid over a binary fibrotic 3D geometry of A) a non-inducible animal, and B) an inducible animal. C) Fibrotic regions in the inducible animals had a larger fiber direction heterogeneity score indicating increased fiber disarray.

$30.7 \pm 0.2\%$, $p < 0.001$). Fiber disarray results are illustrated in Figure 2.

From the tissue samples, the percentage of fibrosis found in the labeled fibrotic regions of the two groups was identical. However, the underlying fibrotic architecture illustrated differences between the two groups. The inducible groups had an increase in obstructive fibrotic strands (34.9 ± 18.9 fibers vs 19.0 ± 6.7 fibers, $p < 0.001$). The obstructive fibrotic strands had greater angle difference from the underlying tissue fiber direction in the inducible animals compared to the non-inducible animals ($16.2 \pm 18.0^\circ$ vs $10.7 \pm 14.9^\circ$, $p < 0.001$). Histology samples are shown in Figure 3.

4. Discussion

Fibrosis has been linked with an increased incidence of AF. Previous works have shown that fibrotic regions affect functional characteristics, leading to slowed conduction and altered conduction direction compared to healthy regions of the atria.[3, 9] The main results of this study illustrate that there are differences in fibrotic architecture. Structural differences in fibrosis may impact conduction differently. The heterogeneity in the fibrotic regions high-

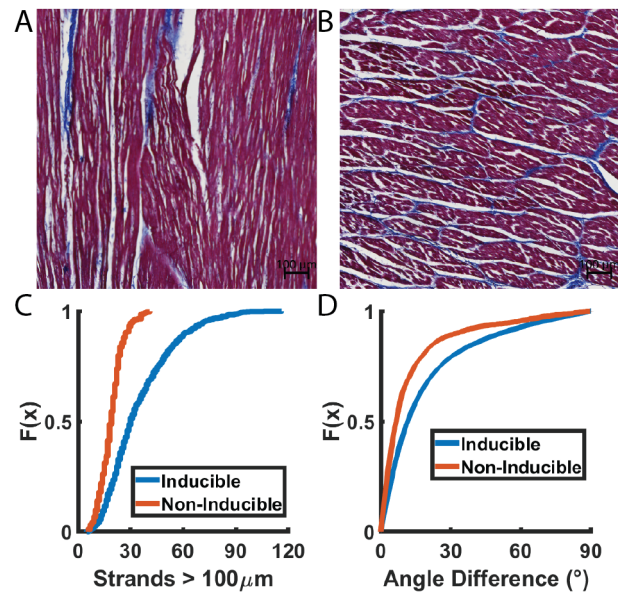


Figure 3. Fibrotic architecture differences within the fibrotic regions of the inducible and non-inducible animals. Masson's Trichrome stain in A) inducible animal and B) non-inducible animal. Notice the differences in fibrotic architecture with fibrotic strands interrupting the tissue fiber connections in the inducible animal sample. C) There are more obstructive strands per mm in the fibrotic regions of the inducible group. D) There is a larger angle difference between the obstructive fibrotic strands and the tissue fiber.

lights the complexity of how structural remodeling may lead to cardiac arrhythmias.

Though atrial fibrosis is associated with AF, in a cohort of transgenic goats with increased cardiac fibrosis, not every goat is inducible for AF, suggesting the simple presence of fibrosis does not lead to AF. Fibrosis may provide a substrate that allows AF to sustain, but functional and structural differences within the fibrotic regions of the atria suggest the presence of heterogeneity within the underlying fibrotic architecture. Variation within the fibrotic regions would lead to the variable arrhythmogenic potential of the substrate.

There is increased structural isotropy in animals that sustained AF. The histology samples revealed increased obstructive fibrotic strands in the inducible animals. These fibrotic strands had a greater angle deviation from the tissue fibers, suggesting that the fibrotic strands were interrupting the longitudinal direction of the fiber. Conduction preferentially flows along the longitudinal direction of tissue fibers, but with roadblocks along the direction due to fibrosis, a zig-zagging pattern may emerge to circumvent the fibrotic obstacles. Zig-zagging conduction pattern may have led to the lower measured conduction anisotropy when calculated on the underlying fiber direction

Future works evaluating how different fibrotic architectures affect conduction propagation and lead to a sustain-

able arrhythmia may shed light on how fibrosis may lead to AF. Improved spatial resolution, evaluating the conduction properties at a cell-cell level through different fibrotic architectures, may confirm the hypothesis of increased zig-zagging conduction patterns in fibrotic regions that have increased obstructive fibrosis. Regions with fibrotic strands interrupting tissue fibers may lead to a uni-directional block and a rotor initiating. Identifying these regions may allow us to identify how fibrosis provides a substrate for AF to sustain.

Computational models have been used extensively to evaluate cardiac arrhythmias; however, accurately simulating atrial fibrillation remains a work in progress. Many models alter the conductivity parameters of the fibrotic regions, but the resulting conductivity is homogeneous throughout the fibrotic regions. A potential improvement to these models is to add variation and heterogeneity to the conductivity of the fibrotic regions.

5. Conclusion

There are underlying fibrotic differences between inducible and non-inducible transgenic goats, suggesting complexity in the fibrotic architecture. The underlying fibrotic architecture may have an important role in determining inducibility and sustainability of AF. When simulating an atrial arrhythmia, it is crucial to consider introducing heterogeneity within the fibrotic regions of the model.

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