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1 **Morphological substrates for atrial arrhythmogenesis in a heart with atrioventricular**
2 **septal defect**

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Abstract

Due to advances in corrective surgery, congenital heart disease has an ever growing patient population. Atrial arrhythmias are frequently observed pre- and post-surgical correction. Pharmaceutical antiarrhythmic therapy is not always effective, therefore many symptomatic patients undergo catheter ablation therapy. In patients with atrioventricular septal defects (AVSD), ablation therapy itself has mixed success; arrhythmogenic recurrences are common, and because of the anatomical displacement of the atrioventricular node, 3-degree heart block post-ablation is a real concern. In order to develop optimal and safe ablation strategies, the field of congenital cardiac electrophysiology must combine knowledge from clinical electrophysiology with a thorough understanding of the anatomical substrates for arrhythmias.

Using image-based analysis and multi-cellular mathematical modelling of electrical activation, we show how the anatomical alterations characteristic of an AVSD serve as arrhythmogenic substrates. Using ex-vivo contrast enhanced micro-computed tomography we imaged post-mortem the heart of a 5 month old male with AVSD at an isometric spatial resolution of 38 μm. Morphological analysis revealed the 3D disposition of the cardiac conduction system for the first time in an intact heart with this human congenital malformation. We observed displacement of the compact atrioventricular node inferiorly to the ostium of the coronary sinus. Myocyte orientation analysis revealed that the normal arrangement of the major atrial muscle bundles was preserved but was modified in the septal region. Models of electrical activation suggest the disposition of the myocytes within the atrial muscle bundles associated with the 'fast pathway', together with the displaced atrioventricular~~AV~~ node, serve as potential substrates for re-entry and possibly atrial fibrillation.

This study used archived human hearts, showing them to be a valuable resource for the mathematical modelling community, and opening new possibilities for the investigations of arrhythmogenesis and ablation strategies in the congenitally malformed heart.

Introduction

The competency and success of corrective surgery is ever improving, as a result congenital heart disease has an ever growing patient population, with adults now outnumbering children (Khairy, 2008). Despite this, atrial arrhythmias are frequently observed pre- and post-surgical correction. Patients with atrioventricular septal defect (AVSD) or atrioventricular canal defect (AVCD) have a common atrioventricular connection, this occurs due to incorrect fusion of the endocardial cushions with the atrial septum and muscular ventricular septum (Anderson, Baker, Yen Ho, Rigby, & Ebels, 2008; Anderson, Ho, & Becker, 2000). Preoperative electrophysiological studies of AVSD patients have shown cases of atrioventricular re-entrant tachycardia (Khairy, Mercier, Dore, & Dubuc, 2007), atrial fibrillation (Daliento et al., 1991; Khairy et al., 2006) and supra-Hisian first degree AV block, and confirm inter-nodal conduction delay in the majority of patients (Fournier, Young,

73 Garcia, Tamer, & Wolff, 1986). Persistent AV block is present in up to 7% of patients in the
74 immediate post-operative period and approximately 2% on follow up (Boening et al., 2002;
75 Daliento et al., 1991), with atrial fibrillation or flutter noted in 5% of patients after surgical
76 repair (Daliento et al., 1991; Vetter & Horowitz, 1982). Many symptomatic patients undergo
77 catheter ablation therapy with varying success, arrhythmogenic recurrences are common.
78 During ablation therapy the interventional cardiologist will target the major muscle bundles
79 believed to be responsible for the inter-nodal conduction disturbance. These bundles have
80 been described previously based on their anatomical appearance and the alignment of the
81 myocyte chains within them (James, 1963; Merideth & Titus, 1968; Sanchez-Quintana, Wyn
82 Davies, Yen Ho, Oslizlok, & Anderson, 1997). More recently these pathways have been
83 described based on their electrophysiology using optical mapping, and are described in the
84 context of the so-called dual pathway physiology (George et al., 2017; Hucker, Fedorov,
85 Foyil, Moazami, & Efimov, 2008; Mani & Pavri, 2014). The pathways are termed the 'slow'
86 and 'fast' pathways; in the healthy heart the 'fast' pathway is the dominant conduction
87 pathway between the sinus node and atrioventricular node. Anatomically the fast pathway
88 courses the anterior-superior aspect of the inter-atrial septum and is associated proximally
89 with the terminal crest and distally with the transitional cells surrounding the compact AV
90 node (George et al., 2017; Mani & Pavri, 2014) (Figure 1A). Conversely, the 'slow' pathway
91 has a less direct course, it runs inferior to the coronary sinus ostium and fossa ovale, and is
92 associated with the flutter isthmus and the inferior nodal extension. In AVSD the
93 atrioventricular node is displaced. The compact atrioventricular node no longer lies at the
94 apex of the triangle of Koch (Figure 1A), but in a posterior-inferior position, anterior to the
95 ostium of the coronary sinus at the point where the posterior-inferior rims of muscular
96 ventricular and atrial septa join (Moorman, de Jong, Denyn, & Lamers, 1998) (Figure 1B).
97 This inevitably changes the anatomical course of the 'fast' and 'slow' pathways (Figure 1B).
98 Conduction disturbances in AVSD patients are associated with prolonged inter-nodal
99 conduction times and numerous conduction disturbances (Fournier et al., 1986; Jacobsen,
100 Gillette, Corbett, Rabinovitch, & McNamara, 1976; Khairy et al., 2006; Waldo, Kaiser,
101 Bowman, & Malm, 1973), presumably because the inter-nodal muscle bundles are distorted
102 or modified as they course the atria (Waldo et al., 1973).

103 Inter-nodal conduction is thus dictated by the location of the nodal tissues and the muscle
104 bundles connecting them. In order to develop optimal and safe ablation strategies for
105 congenitally malformed hearts, the field of congenital cardiac electrophysiology requires an
106 integration of clinical electrophysiology with a thorough understanding of the anatomical
107 substrates for arrhythmias. Guided by the available clinical electrophysiological data we
108 hypothesise that anatomical displacement of the compact atrioventricular node and
109 modification of the dual pathway physiology act as substrates for arrhythmogenesis in AVSD
110 patients. We use image data acquired by micro-computed tomography (micro-CT), as
111 described previously (Stephenson et al., 2017; Stephenson et al., 2012), to extract myocyte
112 orientation and to identify the 3D disposition of the nodal tissue for the first time in an intact
113 heart with AVSD. This information is then incorporated into electrophysiologically accurate
114 mathematical models of electrical activation to assess the influence of these anatomical
115 alterations on inter-nodal conduction. This study also demonstrates the suitability of long

116 term stored archived human hearts as a resource for the mathematical modelling community
117 in investigations of arrhythmogenesis in the congenitally malformed heart.

118

119 **Methods**

120 *Ethical approval statement*

121 We obtained NHS ethical approval to scan congenitally malformed samples from the Alder
122 Hey archive in Liverpool UK. Samples had been consented and placed in the archive in the
123 1970s.

124 *Sample preparation*

125 We chose a sample from the archive free of clotted blood, and probably perfused via the
126 coronary circulation prior to fixation. The sample was from a male who died aged 5 months,
127 and has been in storage for approximately 50 years since the 1970s. The heart dimensions;
128 max length ~70 mm, max width ~55 mm. The sample was prepared for scanning by
129 immersion in 3.75% iodine/potassium iodide (I_2KI) in PBFS for two weeks, refreshing the
130 solution at one week (Stephenson et al., 2017). Iodine molecules are progressively and
131 differentially absorbed by the tissues, permitting discrimination of fat, working myocardium,
132 conducting tissues, and fibrous tissue.

133

134 *Scanning*

135 The sample was scanned in the Nikon Metris XTEK 320 kV Custom Bay at the Manchester
136 X-Ray Imaging Facility, University of Manchester, as previously described by Stephenson et
137 al., (2012 and 2017). Prior to scanning the sample was drained and rinsed in saline to remove
138 excess contrast agent. Plastic wrap provided containment of the tissue, and maintained the
139 original shape of the sample. The heart was immobilized in a plastic tube to reduce
140 movement during the imaging process. Scans were acquired with an X-ray energy of ~95 kV.
141 360° scans were performed and data was collected from 3142 projections. A tungsten target
142 was used for all scans, with a 0.25 mm aluminium filter. Total scan times were approximately
143 50 minutes. Data was reconstructed using filtered back-projection, resulting in tomographic
144 image data with an isotropic voxel size of $38.5 \times 38.5 \times 38.5$ μm. After scanning, the sample
145 was placed back in to formaldehyde solution to allow passive removal of the iodine.

146 *Image analysis*

147 The datasets were examined using Amira (5.3.3) and analysed using objective semi-
148 automatic segmentation methods as described previously (Jarvis & Stephenson, 2013;
149 Stephenson et al., 2017). Muscle bundles associated with the slow and fast pathways along
150 with the terminal crest and common valve annulus were segmented based on the ability to
151 visualise and trace the longitudinal chains of myocyte in the individual muscle bundles using
152 the micro-CT image data. The specialised tissues of the cardiac conduction system were
153 segmented based on their differential attenuation. The electrophysiological block zone, a

154 region of slow conductance between the sinus node and atrial septum, was subjectively
155 placed based on previous representations (Boyett, Honjo, & Kodama, 2000). Myocyte
156 orientation was extracted from the micro-CT data using eigen analysis of the 3D structure
157 tensor as described previously (Ni et al., 2013). To generate myocyte orientation files the raw
158 data was first down-sampled to a spatial resolution of 0.15 mm.

159 *Modelling*

160 To generate a geometrical model for the modelling protocols the raw data was down-sampled
161 to an isotropic spatial resolution of 0.15 mm, which is close to the length of atrial myocytes.
162 Virtual suturing of the dissected borders was performed prior to modelling, such regions were
163 assigned atrial electrophysiological characteristics. Muscle bundle and whole atria electrical
164 activation was modelled using the Coleman-Ni-Zhang (CNZ) model (Ni et al., 2017). In this
165 study cells of the conduction system and the segmented muscle bundles were all assigned as
166 'CT' type. The cells of the atrial working myocardium were assigned as 'RA' type. Cells in
167 the region labelled as the 'block zone' were assigned as 'RA' type but with reduced
168 excitability, this was achieved by reducing their calcium and sodium conductance to 50%.
169 The diffusion parameters were set to a ratio of 8:1 (along the myocyte chain:perpendicular to
170 the myocyte chain). Diffusion coefficients and spatial resolution gave a conduction velocity
171 of 68.2 cm/s for the RA cells. This is within the range of (70.2 +/- 9.9) cm/s measures
172 experimentally in RA cells (Kojodjojo, Kanagaratnam, Markides, Davies, & Peters, 2006). A
173 series of external stimuli with an amplitude of 20 pA/pF and a duration of 2 ms were applied
174 to the sinus node cells in the standard protocols. At fast pacing rates, stimuli with an
175 amplitude of 40 pA/pF and a duration of 4 ms were implemented. During the pacing
176 protocols various S1-S2 intervals were investigated, these ranged from 250 ms to 400ms.

177

178 **Results**

179 *Morphological analysis by micro-computed tomography*

180 The contrast enhanced micro-CT data allowed fast and unequivocal classification of the
181 congenital malformation. We confirmed the heart to have an atrioventricular septal defect
182 with common atrioventricular junction and aligned atrial and muscular ventricular septa
183 (Figure 2). This heart thus exhibits a 'complete defect'.

184 Contrast enhancement permitted discrimination of multiple tissue types based on their
185 differential attenuation of the x-ray source. As a result of differential iodine absorption; fat,
186 myocardium, nodal tissues, and connective tissue presented decreasing voxel values
187 respectively (Figure 3). The sinus node was located as a low attenuating (low voxel values)
188 area in the intercaval region (Figure 1, 2 and 3). The sinus node was seen to give off complex
189 projections into the surrounding working myocardium, with a less pronounced paranodal
190 region than that which is seen in the adult heart (Figure 3B). The compact atrioventricular
191 node was notably displaced from its usual position at the apex of the triangle of Koch. The
192 node was found in a posterior-inferior position anterior to the ostium of the coronary sinus at

193   the point where the posterior-inferior rims of the muscular ventricular and atrial septa join,
194   and was therefore housed in the inferior nodal triangle (Figures 1-4). The atrioventricular
195   conduction axis (AVCA) and the proximal aspects of the right and left bundle branches could
196   also be identified based on their differential attenuation (Figures 3D and 4). The conduction
197   axis was seen to take a long and tortuous path across the crest of the muscular ventricular
198   septum, with the proximal connection between the compact node and the axis appearing quite
199   tenuous. The sinus node and atrioventricular compact node could be identified objectively in
200   both the micro-CT image data (Figure 3) and the derived volume renderings (Figure 1). This
201   is the first time the 3-dimensional disposition of the cardiac conduction system has been
202   presented in a heart with AVSD.

203   It was apparent the heart had undergone attempted correctional surgery, namely the
204   implantation of a surgical patch. This patch itself and the accompanying pledgets and suture
205   lines could be identified in the micro-CT data (Figure 3C and D), and subsequently
206   segmented and presented in 3-dimensions (Figures 2 and 4). The patch had been attached
207   superiorly at the free inferior margin of the atrial septum, which itself appeared hypoplastic.
208   Inferiorly the pledgets and suture lines were placed deep into the right-hand aspect of the
209   muscular ventricular septum. The sutures appeared to pass directly through the nodal tissue,
210   particularly the right bundle branch (Figure 3D and 4).

211   The high resolution micro-CT data allowed the major muscle bundles of the atria to be
212   identified and separated objectively based on their relatively parallel myocyte orientation.
213   The terminal crest, Bachmann's bundle, common valve annulus and the bundles associated
214   with the 'slow' and 'fast' pathways were segmented (Figures 5 and 6). These bundles
215   collectively formed a continuous 'circuit' (Figures 5 and 6). Note the distal aspect of the
216   'fast' pathway showed a continuous connection with the common valve annulus and a
217   distinct muscle bundle traversing the atrial septum (Figure 1B, 5 and 6: red dotted lines). The
218   mean orientation of the myocyte chains could be appreciated by following longitudinal
219   features in volume renderings (Figure 1, 5 and 6) and in the micro-CT image data (Figure
220   3B). Myocyte orientation analysis (see methods for details) confirmed that the mean
221   orientation of the myocyte chains followed the long axis of these identified muscle bundles.

Mathematical modelling

223   NB: When describing the modelling results in the AVSD heart we use the term 'slow' and
224   'fast' pathway based on the traditional identification of their anatomical position in the
225   normal human heart, this is not a reflection of their conduction time.

226   We performed mathematical modelling of the wave of electrical depolarisation in both the
227   isolated muscle bundles and the whole atria. We used a multi-cellular approach, with
228   different models used for the sinus node, block zone, muscle bundles, and the atrial
229   myocardium (see methods). The results of the myocyte orientation analysis were also
230   incorporated into the models by allowing for faster conduction in the long axis of the
231   myocytes than in the orthogonal directions (anisotropic conduction).

232 Activation maps (comprising isochrones) of the isolated muscle bundles showed that the
233 fastest route to the atrioventricular compact node in this heart was via the 'slow' pathway
234 (Figures 5 and 6). This is also clearly illustrated in Ssupplementary video 1. The 'fast'
235 pathway connects with the compact node via the common valve annulus and a distinct septal
236 muscle bundle traversing the atrial septum. Activation via the septal bundle arrived at the
237 node 5-10 milliseconds after the 'slow' pathway (Figures 5 and 6). The results therefore
238 reflect a switch or flipping of the usual dual pathway physiology. The valve annulus provided
239 the slowest route towards the compact node, and its activation was annihilated by stimulation
240 via the 'slow' and 'fast' pathways in an anti-clockwise direction (Supplementary video 1).
241 These results were not affected by the presence or absence of the 'block zone'.

242 Whole atrial modelling showed synchronous activation of the right and left atrial appendages
243 and inter-atrial conduction preferentially via Bachmann's bundle. The results described above
244 for the conspicuous muscle bundles were mirrored when modelling the whole atria, with the
245 fastest route to the atrioventricular compact node seen to be via the 'slow' pathway (Figure 7
246 and Ssupplementary video 2). Figure 7 suggests the 'fast' pathway would be the preferential
247 pathway to the compact node were the node housed in the 'normal' location (Figure 7 B,C
248 and D- red asterisk). Pacing of the whole atria with a 400 millisecond stimulus interval
249 brought about normal sequential atrial activation. S2 intervals less than 300 milliseconds
250 brought about atrial conduction block, with stimulus of the sinus node failing to elicit
251 activation of the whole atria. In these scenarios the stimulus to atrial activation ratio
252 approached 2:1. An S2 interval of 300 milliseconds did, however, elicit atrial activation, but
253 preferential activation of the compact node was no longer via the 'slow' pathway. Preferential
254 conductance and subsequent activation of the nodal region was provided by the 'fast'
255 pathway (Figure 8 B and C). Nodal activation was followed by retrograde propagation up the
256 'slow pathway' (Figure 8C). As a result the muscle bundles associated with 'fast' pathway
257 emerged from their refractory period before those of the 'slow' pathway (Figure 8D). The
258 pacing data presented in Figure 8 is presented as an animation in Ssupplementary video 3.

259

Discussion

261 In this study we show that contrast enhanced micro-CT is an effective non-destructive
262 method for producing high-resolution, high-fidelity, 3-dimensional images of archived
263 human hearts. From these images the 3-dimensional disposition of the cardiac conduction
264 system and the complex arrangement of the myocyte chains can be resolved and quantified.
265 To the best of our knowledge this is the first time such data has been presented for a heart
266 with an AVSD. This high-resolution micro-anatomical data was then used to inform
267 mathematical models of electrical activation, offering a potential stepwise change in the
268 structural fidelity of such models. The resultant simulations are comparable to *in-vivo* clinical
269 assessment of electrophysiology in AVSD patients, suggesting this is a viable technique for
270 the investigation of arrhythmogenesis in congenitally malformed hearts *ex-vivo*.

271 *The competencies of micro-computed tomography*

The nature of micro-CT data means that the morphological structure of this precious archived sample is forever preserved. This data is digital and thus will not degrade over time, and can be easily distributed and visualised using open source software. Thus anatomists, surgeons, cardiologists, engineers, and teachers can easily make use of this new information.

The micro-CT data allowed for fast diagnosis and classification of the defect. Virtual histology (Figure 3) and virtual dissection (Figures 1 and 2) can be performed rapidly and non-destructively in an infinite number of planes. This has clear advantages over traditional destructive, laborious, and error prone techniques such as histology and blunt dissection. As described previously (Stephenson et al., 2017; Stephenson et al., 2012), contrast enhancement allowed the specialised cells of the cardiac conduction system to be resolved independent of the surrounding working myocardium and connective tissue. The disposition of the nodal tissues described in the present study is consistent with previous anatomical accounts of hearts with AVSD using traditional techniques (Anderson et al., 2000). Consistent with previous accounts in the adult human heart (Boyett et al., 2000; Fedorov et al., 2010; Sánchez-Quintana et al., 2005; Stephenson et al., 2017), the sinus node was irregular in shape and occupied a large portion of the inter-caval region, and was seen to give off complex projections into the surrounding myocardium. The sinus node in the AVSD heart did however appear to have a less pronounced paranodal area compared with the adult (Chandler et al., 2011; Stephenson et al., 2017). The nature of the defect and the posterior-inferior displacement of the compact atrioventricular node made for an elongated AVCA, this has been described previously, and is thought to contribute to the prevalence of atrioventricular node block in these patients (Anderson et al., 2008; Anderson et al., 2000; Feldt, Dushane, & Titus, 1970).

In the present study, and previously (Aslanidi et al., 2012; Ni et al., 2013; Stephenson et al., 2017), we have demonstrated how myocyte orientation can be extracted from high-resolution micro-CT data. Extraction of myocyte orientation is imperative to accurate modelling of cardiac electrical activation. Conduction is known to be faster along a cardiomyocyte chain's longitudinal axis than across its short axis (Spach & Kootsey, 1983). The course of the cardiomyocyte chains and their aggregation into distinguishable muscle bundles, therefore, plays a crucial role in inter-nodal conduction. This is highlighted in modelling data presented in the current study (Figures 5-8), and illustrates the importance of the whole heart high-resolution data presented here.

Substrates for arrhythmogenesis in a heart with AVSD

NB: When describing the modelling results in the AVSD heart we use the term 'slow' and 'fast' pathway based on their anatomical position in the normal human heart, this is not a reflection of their conduction time.

The simulations of atrial activation produced in the present study show preferential activation of the compact atrioventricular node via the 'slow' pathway (Figures 5,6,7). This flipping of the dual pathway physiology is consistent with previous *in-vivo* three-dimensional electroanatomic mapping studies, in which the slowest pathway was located superior to

AVCA, while the fastest pathway was identified posterior-inferior to the compact node (Khairy & Balaji, 2009; Khairy et al., 2007). The arrangement is best observed in the right hand and left hand views shown in Ffigure 5. This phenomenon is not surprising considering the displacement of the compact node implies a physical shortening of the 'slow' pathway and a concomitant lengthening of the 'fast' pathway. In this regard, we show how the distal aspect of the 'fast' pathway is continuous with a septal muscle bundle and the common valve annulus. Modelling data suggests conduction along the valve annulus is slow and is annihilated by the slow pathway (Supplementary videos 1 and 2). Tthe preferential route for the 'fast' pathway is, therefore, via the septal bundle. Our data therefore supports the suggestion of Waldo and associates that the middle and anterior (corresponding to the 'fast' pathway) inter-nodal pathways may become distorted or modified due to the septal defect (Waldo et al., 1973).

The area anterior-inferior to the fossa ovale, which in the normal heart houses a distinct muscle bundle and the region of the inferior nodal extension, was seen to be hypoplastic. This suggests this region is not a viable route, and that inter-nodal conduction runs posterior-superior to the fossa ovale via the septal bundle. This, therefore, supports patch placement at the inferior free border of the atrial septum in AVSD hearts. On the other hand, patch placement in the ventricle is hindered by the need to attach the patch to the right hand aspect of the muscular ventricular septum in order to close the defect. Fournier and associates observed right bundle branch block in 19 of 25 postoperative patients (Fournier et al., 1986). Right bundle branch block has historically been a problem in AVSD patients and the necessary placement of pledgets and sutures in the current sample demonstrate the challenge facing the reconstructive surgical team (Ffigure 3D and 4). In this regard micro-CT data has potential implications in the planning of corrective surgery and ablation therapy, pathological reporting, and for investigations into the history of surgical approaches.

Retrograde atrial activation via the fast pathway has been observed previously in AVSD patients (Khairy et al., 2007), and in this case cryomapping of the slow pathway can relieve the accompanying atrioventricular re-entry tachycardia (AVNRT). In the present study atrial pacing using a S2 interval of 300 milliseconds elicited whole atrial activation, but preferential activation of the compact node was no longer via the 'slow' pathway. Preferential conductance and subsequent activation of the nodal region was provided by the 'fast' pathway (Figure 8 B, C and Ssupplementary video 3). Nodal activation was then followed by retrograde propagation up the 'slow' pathway (Figure 8C and Ssupplementary video 3). As a result the muscle bundles associated with 'fast' pathway were seen to leave their refractory period before those of the 'slow' pathway (Figure 8D and Ssupplementary video 3). In this setting the dual pathway physiology therefore becomes desynchronised which could perpetuate both typical and atypical AVNRTs. This finding also provides reasoning for other electrical disturbances observed clinically, such as slow inter-nodal conduction, atrial fibrillation and atrioventricular block (Daliento et al., 1991; Khairy et al., 2006). Furthermore, the data provides evidence to support ablation of the slow pathway in this setting.

Our findings confirm that displacement of the compact atrioventricular node and the accompanying structural modification of the dual pathway physiology provides morphological substrates for arrhythmogenesis in hearts with AVSD.

Future perspectives

The methodologies and concepts presented in the current study provide the opportunity to investigate, and potentially resolve, controversies regarding the anatomical substrates for inter-nodal conduction (Anderson, Ho, Smith, & Becker, 1981; Hucker et al., 2008; Sanchez-Quintana et al., 1997). Future studies using this dataset could include atrio-ventricular and whole heart modelling to investigate substrates and ablation strategies for ventricular tachycardia, atrioventricular block, and bundle branch block, all of which are frequently observed in this defect (Daliento et al., 1991; Khairy, 2008; Khairy & Balaji, 2009). Furthermore, there are many other cardiac congenital malformations that are associated with specific electrical disturbances and arrhythmias. This study is a 'proof of concept', opening the door for wide-scale investigation of arrhythmogenesis by topographical micro-anatomy combined with numerical simulation of electrical activity in the congenitally malformed heart.

Study limitations

We recognise that this study lacks an age-matched healthy control to validate our findings, but such a sample would be extremely difficult to obtain. The major limitation of this study is the need to downsample the high-resolution information-rich micro-CT data into a form which is computationally manageable. The large file size, in this case ~10 GB, and the fine structural details, make the integration of such data into mathematical models, computationally and theoretically difficult. This, however, highlights a new research challenge for the modelling and engineering community. While providing new challenges, high resolution micro-CT data provides a step change in the quality of structural geometries available to groups working on mathematical models of cardiac depolarisation.

Acknowledgments

Conflict of Interest Statement

The authors declare no conflict of interest.

Funding statement

Data availability statement

The datasets for this manuscript are not publicly available because: [this patient data is sensitive and ethical approval is acquired on an individual basis]. Requests to access the datasets should be directed to [Dr Robert Stanley Stephenson, email: robert.stephenson@clin.au.dk].

Author Contributions Statement

Acquisition of ethical approval for the study (JJ, CJ, RG).

Sample preparation (JJ, RS) and data acquisition (RS, JJ, TL).

Data analysis and production of figures (JN, RS, HZ).

Writing (RS) and editing of manuscript (JN, CJ, RG, JZ, HZ, JJ).

References

Anderson, R. H., Baker, E. J., Yen Ho, S., Rigby, M. L., & Ebels, T. (2008). The morphology and diagnosis of atrioventricular septal defects. *Cardiology in the Young, 1*(4), 290-305. doi: 10.1017/S1047951100010362

Anderson, R. H., Ho, S. Y., & Becker, A. E. (2000). Anatomy of the human atrioventricular junctions revisited. *The Anatomical Record, 260*(1), 81-91. doi: doi:10.1002/1097-0185(20000901)260:1<81::AID-AR90>3.0.CO;2-3

Anderson, R. H., Ho, S. Y., Smith, A., & Becker, A. E. (1981). The internodal atrial myocardium. *The Anatomical Record, 201*(1), 75-82. doi: doi:10.1002/ar.1092010110

Aslanidi, O., Nikolaidou, T., Zhao, J., Smaill, B., Gilbert, S., Jarvis, J., . . . Zhang, H. (2012). Application of Micro-Computed Tomography with Iodine Staining to Cardiac Imaging, Segmentation and Computational Model Development. *Medical Imaging, IEEE Transactions on, PP*(99), 1-1. doi: 10.1109/tmi.2012.2209183

Boening, A., Scheewe, J., Heine, K., Hedderich, J., Regensburger, D., Kramer, H. H., & Cremer, J. (2002). Long-term results after surgical correction of atrioventricular septal defects.

425   *European Journal of Cardio-Thoracic Surgery, 22*(2), 167-173. doi: 10.1016/S1010-
426   7940(02)00272-5

427 Boyett, M. R., Honjo, H., & Kodama, I. (2000). The sinoatrial node, a heterogeneous pacemaker
428   structure. *Cardiovascular Research, 47*(4), 658-687. doi: 10.1016/s0008-6363(00)00135-8

429 Chandler, N., Aslanidi, O., Buckley, D., Inada, S., Birchall, S., Atkinson, A., . . . Dobrzynski, H. (2011).
430   Computer Three-Dimensional Anatomical Reconstruction of the Human Sinus Node and a
431   Novel Paranodal Area. *The Anatomical Record: Advances in Integrative Anatomy and
432   Evolutionary Biology, 294*(6), 970-979. doi: 10.1002/ar.21379

433 Daliento, L., Rizzoli, G., Marchiori, M. C., Buja, G., Milanesi, O., Valente, S., . . . Mazzucco, A. (1991).
434   Electrical instability in patients undergoing surgery for atrioventricular septal defect.
435   *International Journal of Cardiology, 30*(1), 15-21. doi: 10.1016/0167-5273(91)90119-A

436 Fedorov, V. V., Glukhov, A. V., Chang, R., Kostecki, G., Aferol, H., Hucker, W. J., . . . Efimov, I. R.
437   (2010). Optical Mapping of the Isolated Coronary-Perfused Human Sinus Node. *Journal of
438   the American College of Cardiology, 56*(17), 1386-1394. doi:
439   http://dx.doi.org/10.1016/j.jacc.2010.03.098

440 Feldt, R. H., Dushane, J. W., & Titus, J. I. (1970). The Atrioventricular Conduction System in Persistent
441   Common Atrioventricular Canal Defect. *Correlations with Electrocardiogram, 42*(3), 437-444.
442   doi: 10.1161/01.cir.42.3.437

443 Fournier, A., Young, M.-L., Garcia, O. L., Tamer, D. F., & Wolff, G. S. (1986). Electrophysiologic cardiac
444   function before and after surgery in children with atrioventricular canal. *The American
445   Journal of Cardiology, 57*(13), 1137-1141. doi: https://doi.org/10.1016/0002-9149(86)90688-
446   0

447 George, S. A., Faye, N. R., Murillo-Berlioz, A., Lee, K. B., Trachiotis, G. D., & Efimov, I. R. (2017). At the
448   Atrioventricular Crossroads: Dual Pathway Electrophysiology in the Atrioventricular Node
449   and its Underlying Heterogeneities. *Arrhythmia & Electrophysiology Review, 6*(4), 179-185.
450   doi: 10.15420/aer.2017.30.1

451 Hucker, W. J., Fedorov, V. V., Foyil, K. V., Moazami, N., & Efimov, I. R. (2008). Optical Mapping of the
452   Human Atrioventricular Junction. *Circulation, 117*(11), 1474-1477. doi:
453   10.1161/circulationaha.107.733147

454 Jacobsen, J. R., Gillette, P. C., Corbett, B. N., Rabinovitch, M., & McNamara, D. G. (1976). Intracardiac
455   electrography in endocardial cushion defects. *Circulation, 54*(4), 599-603. doi:
456   10.1161/01.cir.54.4.599

457 James, T. N. (1963). The connecting pathways between the sinus node and A-V node and between
458   the right and the left atrium in the human heart. *American Heart Journal, 66*(4), 498-508.
459   doi: http://dx.doi.org/10.1016/0002-8703(63)90382-X

460 Jarvis, J. C., & Stephenson, R. (2013). Studying the Microanatomy of the Heart in Three Dimensions:
461   A Practical Update. *Frontiers in Pediatrics, 1*, 26. doi: 10.3389/fped.2013.00026

462 Khairy, P. (2008). EP challenges in adult congenital heart disease. *Heart Rhythm, 5*(10), 1464-1472.
463   doi: 10.1016/j.hrthm.2008.05.026

464 Khairy, P., & Balaji, S. (2009). Cardiac Arrhythmias In Congenital Heart Diseases. *Indian pacing and
465   electrophysiology journal, 9*(6), 299-317.

466 Khairy, P., Dore, A., Talajic, M., Dubuc, M., Poirier, N., Roy, D., & Mercier, L.-A. (2006). Arrhythmias in
467   adult congenital heart disease. *Expert Review of Cardiovascular Therapy, 4*(1), 83-95. doi:
468   10.1586/14779072.4.1.83

469 Khairy, P., Mercier, L.-A., Dore, A., & Dubuc, M. (2007). Partial atrioventricular canal defect with
470   inverted atrioventricular nodal input into an inferiorly displaced atrioventricular node. *Heart
471   Rhythm, 4*(3), 355-358. doi: 10.1016/j.hrthm.2006.10.012

472 Kojodjojo, P., Kanagaratnam, P., Markides, V., Davies, W., D. , & Peters, N. (2006). Age-Related
473   Changes in Human Left and Right Atrial Conduction. *Journal Of Cardiovascular
474   Electrophysiology, 17*(2), 120-127. doi: doi:10.1111/j.1540-8167.2005.00293.x

475 Mani, B. C., & Pavri, B. B. (2014). Dual Atrioventricular Nodal Pathways Physiology: A Review of
476   Relevant Anatomy, Electrophysiology, and Electrocardiographic Manifestations. *Indian*
477   *pacing and electrophysiology journal, 14*(1), 12-25.
478 Merideth, J., & Titus, J. I. (1968). The Anatomic Atrial Connections Between Sinus and A-V Node.
479   *Circulation, 37*(4), 566-579. doi: 10.1161/01.cir.37.4.566
480 Moorman, A. F. M., de Jong, F., Denyn, M. M. F. J., & Lamers, W. H. (1998). Development of the
481   Cardiac Conduction System. *Circulation Research, 82*(6), 629-644. doi:
482   10.1161/01.res.82.6.629
483 Ni, H., Castro, S. J., Stephenson, R. S., Jarvis, J. C., Lowe, T., Hart, G., . . . Zhang, H. (2013, 22-25 Sept.
484   2013). *Extracting myofibre orientation from micro-CT images: An optimisation study.* Paper
485   presented at the Computing in Cardiology 2013.
486 Ni, H., Whittaker, D. G., Wang, W., Giles, W. R., Narayan, S. M., & Zhang, H. (2017). Synergistic Anti-
487   arrhythmic Effects in Human Atria with Combined Use of Sodium Blockers and Acacetin.
488   *Frontiers in Physiology, 8*(946). doi: 10.3389/fphys.2017.00946
489 Sánchez-Quintana, D., Cabrera, J. A., Farré, J., Climent, V., Anderson, R. H., & Ho, S. Y. (2005). Sinus
490   node revisited in the era of electroanatomical mapping and catheter ablation. *Heart, 91*(2),
491   189-194. doi: 10.1136/hrt.2003.031542
492 Sanchez-Quintana, D., Wyn Davies, D., Yen Ho, S., Oslizlok, P., & Anderson, R. H. (1997). Architecture
493   of the Atrial Musculature In and Around the Triangle of Koch. *Journal Of Cardiovascular*
494   *Electrophysiology, 8*(12), 1396-1407. doi: 10.1111/j.1540-8167.1997.tb01036.x
495 Spach, M. S., & Kootsey, J. M. (1983). The nature of electrical propagation in cardiac muscle.
496   *American Journal of Physiology - Heart and Circulatory Physiology, 244*(1), H3-H22.
497 Stephenson, R. S., Atkinson, A., Kottas, P., Perde, F., Jafarzadeh, F., Bateman, M., . . . Dobrzynski, H.
498   (2017). High resolution 3-Dimensional imaging of the human cardiac conduction system
499   from microanatomy to mathematical modeling. *Scientific Reports, 7*(1), 7188. doi:
500   10.1038/s41598-017-07694-8
501 Stephenson, R. S., Boyett, M. R., Hart, G., Nikolaidou, T., Cai, X., Corno, A. F., . . . Jarvis, J. C. (2012).
502   Contrast Enhanced Micro-Computed Tomography Resolves the 3-Dimensional Morphology
503   of the Cardiac Conduction System in Mammalian Hearts. *PLoS ONE, 7*(4), e35299.
504 Vetter, V. L., & Horowitz, L. N. (1982). Electrophysiologic residua and sequelae of surgery for
505   congenital heart defects. *American Journal of Cardiology, 50*(3), 588-604. doi: 10.1016/0002-
506   9149(82)90328-9
507 Waldo, A. I., Kaiser, G. A., Bowman, F. O., & Malm, J. R. (1973). Etiology of Prolongation of the P-R
508   Interval in Patients with an Endocardial Cushion Defect. *Further Observations on Internodal*
509   *Conduction and the Polarity of the Retrograde P Wave, 48*(1), 19-26. doi:
510   10.1161/01.cir.48.1.19

511

Figure legends

512

513 **Figure 1.** Volume renderings of the atrial cavity of a heart with atrioventricular septal defect
514 (AVSD). (A) indicates the anatomical locations of the slow pathway (green), fast pathway
515 (red), and compact atrioventricular node (*) in the normal human heart superimposed on the
516 AVSD anatomy, viewed from inferior-lateral position. (B) indicates the hypothesised
517 anatomical locations of the slow pathway (green), fast pathway (red), and compact
518 atrioventricular node (*) in a heart with a atrioventricular septal defect. Images derived from
519 micro-CT data. *- location of compact atrioventricular node, CS- coronary sinus, CT-
520 terminal crest, FO- fossa ovale, SN- sinus node, VA- valve annulus. Scale bar represents 3
521 mm.

Figure 2. Long axis volume renderings of a heart with atrioventricular septal defect (AVSD). (A) Anterior 4-chamber view, (B) posterior 4-chamber view, (C) right side two-chamber view, (D) left side two-chamber view. The sinus node is shown in yellow, the atrioventricular conduction axis in green, and the surgical patch in blue. Images derived from micro-CT data. Ao- aorta, AS- atrial septum, AVCA- atrioventricular conduction axis, LV- left ventricle, MS- muscular ventricular septum, PT- pulmonary trunk, RV- right ventricle, SN- sinus node. Scale bar represents 3 mm.

Figure 3. Virtual histology of the cardiac conduction system in a heart with atrioventricular septal defect (AVSD). (A) Volume rendering of the whole heart illustrating the virtual cutting planes used in panels B,C and D. (B) Short axis micro-CT section of the sinus node, (C) two-chamber micro-CT section of the compact atrioventricular node, (D) 4-chamber micro-CT section of the atrioventricular conduction axis. AVCA- atrioventricular conduction axis, CN- compact atrioventricular node, CS- coronary sinus, CT- terminal crest, LV- left ventricle, MS- muscular ventricular septum, RV- right ventricle, SN- sinus node, solid arrow heads- pledget and suture line. Scale bars represents 1 mm.

Figure 4. 3-dimensional rendering of the atrioventricular conduction axis in a heart with atrioventricular septal defect (AVSD). Showing the conduction axis (green) and the surgically placed pledgets and sutures (blue) in anterior (A) and right lateral views (B). Images derived from segmentation of micro-CT data. CN- compact atrioventricular node. Scale bar represents 1 mm.

Figure 5. Inter-nodal conduction through the atrial muscle bundles I. Volume renderings of the atrial muscle bundles in right lateral (A) and left lateral (C) views, the location and direction of the slow pathway (green), and distal aspect of the fast pathway i.e. the septal bundle (red) are indicated by dotted arrows. Panels B and D show the corresponding electrical activation maps. See methods for modelling parameters. BB- Bachmann's bundle, CN- compact atrioventricular node, CT- terminal crest, SN- sinus node, VA- valve annulus. Scale bar represents 3 mm.

Figure 6. Inter-nodal conduction through the atrial muscle bundles II. Volume renderings of the atrial muscle bundles in anterior (A) and inferior (C) views, the location and direction of the slow pathway (green), and distal aspect of the fast pathway i.e. the septal bundle (red) are indicated by dotted arrows. Panels B and D show the corresponding electrical activation maps. See methods for modelling parameters. *- location of compact atrioventricular node, BB- Bachmann's bundle, CT- terminal crest, SN- sinus node, VA- valve annulus. Scale bar represents 3 mm.

Figure 7. Preferential inter-nodal conduction via the 'slow' pathway in the whole atria of a heart with AVSD. (A) Volume rendering of the atrial cavity viewed from the inferior-lateral position. (B) Corresponding isochrone electrical activation map, the direction and position of the slow pathway (green), and distal aspect of the fast pathway (red) are indicated by solid arrows. (C and D) Snapshots taken from the Ssupplementary video 2 showing excitation of the distal aspect of the 'slow' pathway (green) precedes that of the 'fast' pathway (red), pink

indicates activated myocardium, light blue indicates dormant myocardium. See methods for modelling parameters. White*- location of compact atrioventricular node in AVSD heart, Red*- location of compact atrioventricular node in normal heart, CS- coronary sinus, CT- terminal crest, FO- fossa ovale, LAA- left atrial appendage, RAA- right atrial appendage, SN- sinus node, VA- valve annulus. Scale bar represents 3 mm.

Figure 8. Fast pacing elicits retrograde conduction via the 'fast' pathway in the atria of a heart with AVSD. (A-D) Time-lapse snapshots taken from the Ssupplementary video 3 showing preferential inter-nodal conduction via the 'fast' pathway during a atrial pacing protocol (s1-s2 interval 300 ms). Views are comparable to those presented in Ffigure 7. The direction and position of the slow pathway (green), and distal aspect of the fast pathway (red) are indicated by solid arrows. Pink indicates activated myocardium, light blue indicates dormant myocardium. See methods for modelling parameters. White*- location of compact atrioventricular node in AVSD heart, Red*- location of compact atrioventricular node in normal heart, CS- coronary sinus, CT- terminal crest, FO- fossa ovale, LAA- left atrial appendage, RAA- right atrial appendage, SN- sinus node.