



ORIGINAL ARTICLE / *Forensic medicine*

## Evaluation of septal insertion of atrioventricular valves in fetuses by postmortem 4.7 Tesla cardiac MRI: A feasibility study

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### KEYWORDS

Atrioventricular septal defect;  
Postmortem MRI;  
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Linear insertion of atrioventricular valves

### Abstract

**Purpose:** The purpose of this study was to compare non-invasive high-spatial-resolution post-mortem cardiac magnetic resonance imaging (MRI) and autopsy findings for evaluating the septal insertion of atrioventricular valves in fetuses.

**Materials and methods:** Five fetal heart specimens including two normal hearts, one heart with complete atrioventricular septal defect (AVSD) and two hearts with linear insertion of atrioventricular valves (LIAVV; gestational age 17 to 34 weeks) were studied with cardiac MRI using a 4.7 T MRI scanner without sample preparation. Three (3D) and two-dimensional (2D) turbo-RARE (rapid imaging with refocused echoes) sequences in four-chamber and left-ventricular long-axis planes were obtained with a minimal isotropic/in-plane resolution of 156  $\mu\text{m}$ . Nonparametric tests were performed to compare the distance between insertions of medial leaflets of the atrioventricular valves and the inlet/outlet distance ratio between MRI and autopsy findings in normal, complete AVSD and with linear insertion of atrioventricular valves (LIAVV) fetal hearts.

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**Results:** Despite apparent differences between LIAVV/normal hearts, no significant differences were found between differential insertion of medial leaflets and inlet/outlet distance ratios with both techniques. Very good to excellent reliability between both techniques was found for differential insertion (ICC: 87.2%; 95% CI: -21.7%, 99.1%) ( $P=0.963$ ) and inlet/outlet distance ratio (ICC 98.3%; 95%CI: 85.2%, 99.8%) ( $P=0.537$ ) measurements.

**Conclusion:** Postmortem cardiac MRI could replace autopsy for assessing normal or abnormal septal insertion of atrioventricular valves in fetuses without requiring specific preparation of the heart.

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The morphologic diagnosis of abnormal septal insertion of atrioventricular valves in fetal heart specimen requires a precise and additional apex-to-base section to obtain an appropriate section of the crux of the heart, parallel to the diaphragm and perpendicular to the ventricular septum, at the level of the inferior pulmonary veins (mimicking the echocardiographic four-chamber view) [1]. Such a section is required for example to demonstrate the horizontal continuity of the valves in linear insertion of atrioventricular valves (LIAVV) [2,3], which is an echocardiographic sign defined by the absence of offsetting of the two atrioventricular valves on a four-chamber view. This sign is considered one of the hallmarks of atrioventricular septal defects (AVSD) or atrioventricular canal malformations, in which it represents the echocardiographic translation for the commonality of the atrioventricular junction [3,4]. However, when the heart specimen is cut in this way, accurate analysis of the ventricular septum to search for a ventricular septal defect becomes very difficult. It would thus be interesting to replace this special section of the heart by a non-invasive method, allowing thorough examination of the heart without distorting intracardiac structures. Postmortem cardiac magnetic resonance imaging (MRI) could be useful in this setting, as it can provide morphometric data in fetal hearts that allow for diagnostic evaluation of most congenital cardiac abnormalities [5–7].

The purpose of this study was to compare non-invasive high-spatial-resolution postmortem cardiac MRI and autopsy findings for evaluating the septal insertion of atrioventricular valves in fetuses.

## Materials and methods

### Heart selection and preparation

For this feasibility study, five fetal heart specimens were selected from the anatomic collection of the French Reference Center for Complex Congenital Heart Defects in Marie-Lannelongue Hospital. This study was approved by the local research ethics committee. Fetal hearts were classified according to prenatal ultrasound suspected diagnosis and autopsy-proven congenital heart defect: 2 out of 15 normal hearts at 16 to 39 weeks of gestation, 1 out of 21 hearts

with complete AVSD at 13 to 36 weeks of gestation, and 2 out of 32 LIAVV hearts at 15 to 35 weeks of gestation. Heart specimens were preserved in a 10% formalin solution. For MRI, they were rinsed and placed in airtight bags filled with 1 to 2 mL distilled water. Specimens were then placed in a cradle of foam to ensure stability during MRI.

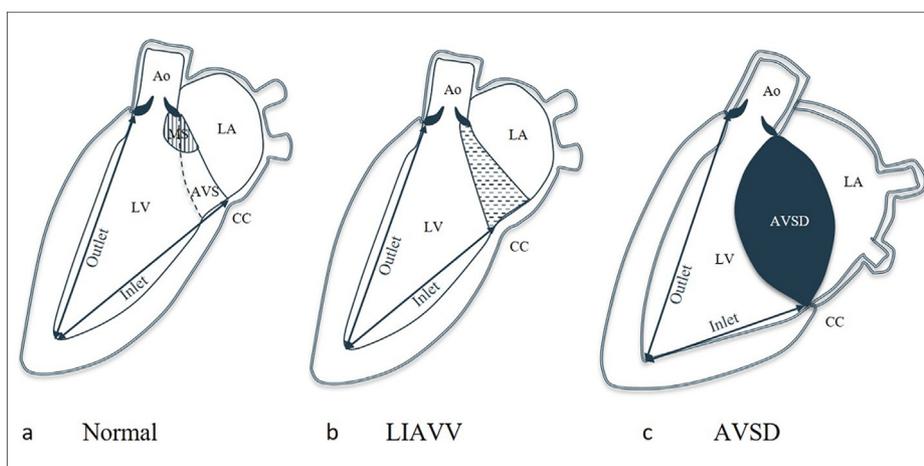
### Postmortem cardiac imaging

MRI of hearts involved use of a 4.7 T MR imager (Biospec® 47/40; Brucker Biospin) containing a quadrature transmit/receive homogeneous coil with a 35 mm inner diameter. Three-dimensional (3D) and two-dimensional (2D) turbo-RARE (rapid imaging with refocused echoes) sequences were optimized for hearts <1 cm in the long-axis (Table 1). Four-chamber, short-axis and left-ventricular long-axis planes were examined at a minimal isotropic or in-plane resolution of 156  $\mu\text{m}$ . The left-ventricular long-axis orientation using MRI was defined by the direction of the vector starting

**Table 1** Imaging parameters for postmortem cardiac MRI.

	2D turbo-RARE	3D turbo-RARE
TE (msec)	44	46
TR (msec)	3500	3500
Averages	4	10
Bandwidth (kHz)	25	50
Field of view (mm)	1337 × 716	1337 × 716
Matrix resolution	256 × 256	224 × 224
Pixel or voxel dimension ( $\mu\text{m}$ )	156 × 156 × 700	156 × 156 × 156
Section thickness (mm)	0.7	N.A.
Acquisition time (sec)	14	600

TE: excitation time; TR: repetition time; 2D: two-dimensional; RARE: rapid imaging with refocused echoes; N.A.: not applicable.



**Figure 1.** Diagrams show the spectrum of atrioventricular septal defects with the left side of the septum on a left-ventricular long-axis plane. a: normal inlet/outlet ratio in a normal fetal heart; b: abnormal inlet/outlet ratio in a fetal heart with linear insertion of the atrioventricular valves; c: disproportion of the inlet/outlet distance ratio in a fetal heart with atrioventricular septal defect (Ao: Aorta; MS: membranous septum; AVS: atrioventricular septum; LA: left atrium; LV: left ventricle; CC: crux cordis).

from the centre of the mitral annulus projecting through the LV apex. One radiologist (E. B.), blinded to fetus status, performed qualitative and quantitative assessment of valve insertion by MRI. The degree of offsetting between the septal insertion of tricuspid and mitral valves was searched for. Quantitative assessment involved measuring the differential insertion of the atrioventricular valves. Moreover, it has been suggested that the inlet measured on a left-ventricular long-axis view of the interventricular septum would appear shorter in specimens with AVSD than in normal hearts (Fig. 1). Therefore, the inlet/outlet distance ratio of the left ventricle was calculated.

### Macroscopic examination

The macroscopic evaluation of the fetal heart was performed by a cardiac morphologist (LH). Special attention was paid to the anatomy of the ventricular septum viewed from the left-ventricular side. In all five specimens, the left ventricle was opened along the septal surface to permit a complete exposure of the septum, sectioned in a base-to-apex plane [2]. The quantitative and qualitative features studied on MRI were compared to autopsy findings.

### Statistical analysis

Quantitative data were expressed as medians and interquartile range (IQR; first quartile [Q<sub>1</sub>] – third quartiles [Q<sub>3</sub>]). Between-groups comparisons were performed using Mann-Whitney rank sum tests, while within-groups comparisons between MRI and autopsy measurements were carried out by means of the Wilcoxon signed-ranks test for paired data. The intraclass correlation coefficient along with its 95% confidence interval was computed to assess the reliability between quantitative measurements obtained by autopsy and MRI, with values greater than 0.80 and 0.90 indicating very good and excellent reliability, respectively. Analyses were performed using Stata v15.1 (StataCorp).

### Results

Five fetal hearts were examined including 2 normal hearts, 1 heart with a complete AVSD and 2 hearts with LIAVV. Gestational age ranged from 17 to 34 weeks (median 27 weeks of gestation; Q<sub>1</sub>, 26; Q<sub>3</sub>, 28). The characteristics of the hearts are summarized in Table 2.

### Qualitative data

The presence of a septal defect was correctly identified with MRI in the case of AVSD. The differential insertion in normal hearts and the continuity between the mitral and tricuspid valves in LIAVV hearts were easily identified on MRI. This qualitative evaluation of the insertion of mitral and tricuspid valves in normal and abnormal hearts on the four-chamber view was similar with MRI and autopsy (Table 2).

### Quantitative data

The distance between the insertion of mitral and tricuspid valves was apparently greater in normal hearts than in LIAVV hearts regardless of the technique of measurement (Fig. 2), with distances estimated by MRI between the insertion of the medial leaflets of the tricuspid and mitral valves of 1.69 and 1.80 mm for normal hearts and 0.10 and 0.82 mm for LIAVV hearts.

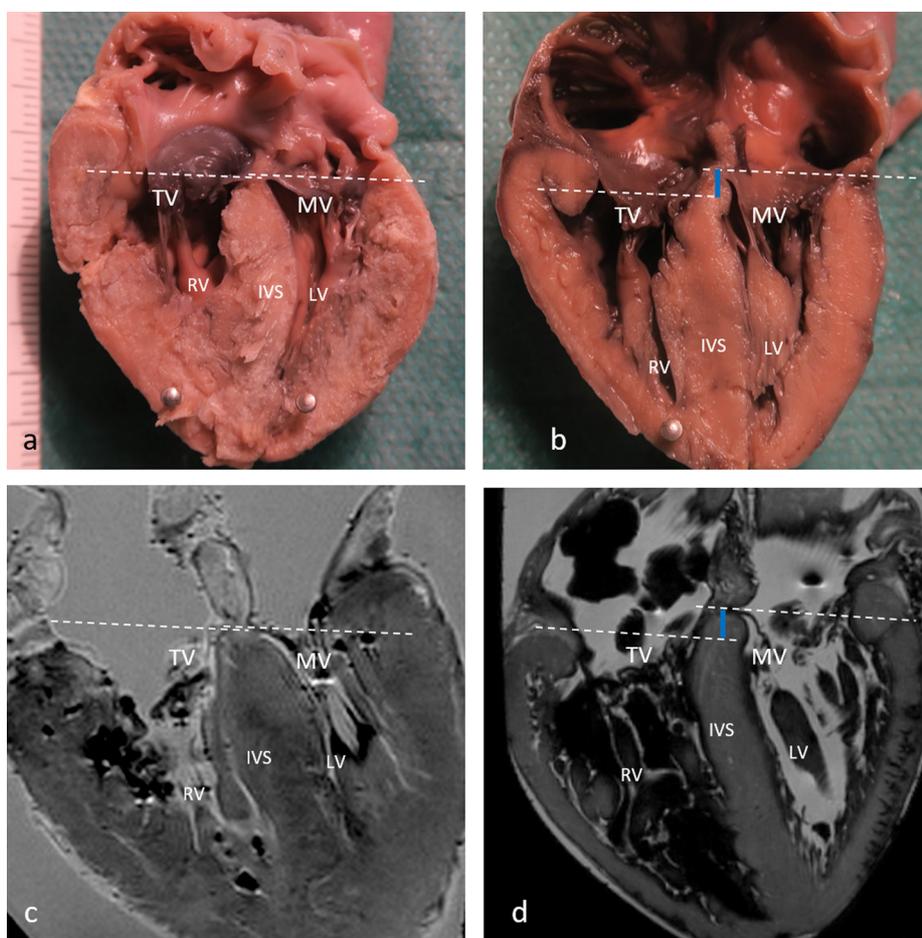
At MRI no significant differences in valves distances were found between LIAVV (median = 0.46 mm; Q<sub>1</sub>, 0.10 mm; Q<sub>3</sub>, 0.82 mm) and normal hearts (median = 1.75 mm; Q<sub>1</sub>, 1.69 mm; Q<sub>3</sub>, 1.80 mm) ( $P=0.121$ ). Similarly, at autopsy no significant differences in valves distances were found between LIAVV (median = 0.07 mm; Q<sub>1</sub>, 0.01 mm; Q<sub>3</sub>, 0.13 mm) and normal hearts (median = 2.17 mm; Q<sub>1</sub>, 1.89 mm; Q<sub>3</sub>, 2.44 mm) ( $P=0.121$ ). Reliability between MRI and autopsy was very good (ICC = 87.2%; 95%CI: -21.7%, 99.1%) and no significant differences were found between MRI and autopsy mean measurements ( $P=0.715$ ).

Inlet/outlet distance ratios were  $\geq 0.9$  in normal hearts,  $\leq 0.81$  in LIAVV hearts and  $<0.50$  in the AVSD heart (Fig. 3).

**Table 2** Sample characteristics and comparison of findings and diagnoses by high-spatial – resolution MRI and macroscopic examination.

Case#	Gestational age (WG)	Chromosomal abnormality	Qualitative assessment				Quantitative assessment				Final diagnosis
			Presence of a defect		Abnormal septal insertion of tricuspid and mitral valves		Tricuspid/mitral septal insertion distance		Inlet-outlet distance ratio		
			MRI	Autopsy	MRI	Autopsy	MRI	Autopsy	MRI	Autopsy	
1	17	Trisomy 21	Yes	Yes	Yes	Yes	NA	NA	0.49	0.45	AVSD
2	28	Trisomy 21	No	No	Yes	Yes	0.10	0.01	0.77	0.81	LIAVV
3	26	Trisomy 13	No	No	Yes	Yes	0.82	0.13	0.80	0.80	LIAVV
4	34	None (poly-malformative syndrome)	No	No	No	No	1.69	2.44	0.90	0.91	Normal
5	27	Trisomy 21	No	No	No	No	1.80	1.89	0.90	0.94	Normal

WG: weeks' gestation; AVSD: atrioventricular septal defect; LIAVV: linear insertion of atrioventricular valve.



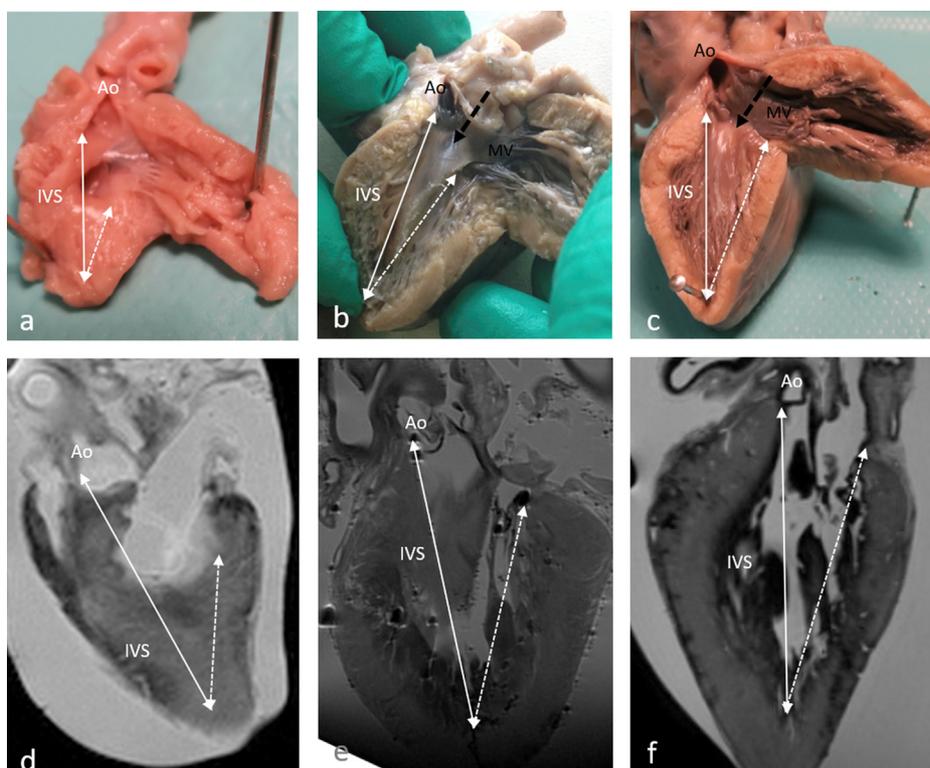
**Figure 2.** Evaluation of the differential insertion of mitral and tricuspid valves on autopsy (a; b: top) and MRI four-chamber views (c; d: bottom) with: linear insertion of atrioventricular valves (LIAVV) (a; c: case 4) and normal insertion (b; d: case 6). In normal heart, the tricuspid valve inserts closer to the cardiac apex compared with the mitral valve (dotted lines). In LIAVV, a horizontal continuity of the septal insertion of mitral and tricuspid valves exists. The distance between the insertion of atrioventricular valves (blue lines) is greater in normal heart compared with LIAVV regardless of the technique of measurement (TV: tricuspid valve; MV: mitral valve; LV: left ventricle; IVS: interventricular septum).

At MRI, no significant differences in inlet/outlet distance ratios were found between LIAVV (median=0.78;  $Q_1$ , 0.77;  $Q_3$ , 0.80) and normal hearts (median 0.90;  $Q_1$ , 0.90;  $Q_3$ , 0.90) ( $P=0.102$ ). Similarly, at autopsy no significant differences in inlet/outlet distance ratios were found between LIAVV (median=0.81;  $Q_1$ , 0.80;  $Q_3$ , 0.81) and normal hearts (median=0.93;  $Q_1$ , 0.91;  $Q_3$ , 0.94) ( $P=0.121$ ). Excellent reliability between both techniques was found for inlet/outlet distance ratios (ICC = 98.3%; 95%CI: 85.2%, 99.8%) ( $P=0.405$ ).

## Discussion

In this preliminary study, very good to excellent concordance was found between MRI and autopsy findings for the evaluation of mitral and tricuspid valves insertion and inlet/outlet distance ratio. These results support the feasibility of evaluating the offsetting of atrioventricular valves with postmortem high-spatial-resolution MRI without specific preparation of heart specimens.

In the normal heart, the tricuspid valve inserts on the ventricular septum closer to the cardiac apex as compared with the mitral valve (normal offsetting of the hinge points of the atrioventricular valves). The abnormal insertion at the same level, of both septal portions of the left and right atrioventricular valves was described echocardiographically for the first time by Seward et al. in a partial atrioventricular canal [8]. This setting has been described in hearts without AVSD and was called decreased or narrowed mitral valve-tricuspid valve distance [9], or LIAVV [2]. Fetopathology examination is the reference method for the diagnosis of abnormal insertion of atrioventricular valves but requires sectioning the heart in a precise apex-to-base plane, particularly for the diagnosis of LIAVV, as described by Fredouille et al. [2]. This section is technically difficult to realize and can result in some cases in misinterpretation in non-expert hands. Moreover, conventional autopsy and dissection are inherently destructive. Once the sample is sectioned in a particular plane, it is no longer possible to reexamine the heart in another, and possibly more appropriate, plane.



**Figure 3.** Evaluation of the inlet (dotted arrow)/outlet (arrow) distance ratio on macroscopic dissection and MRI left sagittal views of the interventricular septum in hearts with (a; d) complete atrioventricular septal defect (case 1), (b; e) linear insertion of atrioventricular valves (LIAVV) (case 3) and (c; f) absence of abnormality (case 5). Note the visibility of losangic membranous septum on macroscopic dissection (b) (dotted black arrows) in LIAVV (IVS: interventricular septum, Ao: aorta, MV: mitral valve).

Postmortem imaging has the advantage of analyzing the heart nondestructively, allowing for acquiring or reconstructing images of the heart in any plane with permanent data storage. Potential imaging modalities for fetal hearts are postmortem ultrasound, micro computed tomography (CT) and MRI. Tuchtan et al. reported the use of postmortem ultrasound for the diagnosis of congenital abnormalities in perinatal death [10]. However, the diagnostic performance of ultrasound regarding cardiac abnormalities was low in this study with a sensitivity of 18.2%. Postmortem cardiac CT requires sample preparation with either brown discoloration resulting from immersion of the heart in a potassium triiodide solution before micro CT [11] or the use of umbilical vein catheterization to obtain whole body postmortem angiography [12]. Thanks to its spontaneous contrast tissue resolution, postmortem cardiac MRI appears as the technique of choice for examining the fetal heart. Recent studies have reported a good accuracy for the detection of congenital heart defects in *in situ* fetal hearts by using 3D cardiac MRI at 1.5 T and 3 T [5,6] and postmortem 2D high-spatial – resolution MRI at 9.4 T [7,13]. However, most overcalls or missed abnormalities with postmortem cardiac fetal MRI were ventricular septal defects and atrial septal defects [6]. We then performed not only a qualitative analysis of the atrioventricular valves septal insertion but also a precise quantitative analysis of the distance between septal insertion of the mitral and tricuspid valves and the inlet/outlet ratio in a left-ventricular long-axis view using postmortem MRI.

First, the distance between septal insertions of the mitral and tricuspid valves was apparently greater for normal hearts as compared with LIAVV hearts on MRI. However, formal testing comparison revealed no statistically significant difference between LIAVV and normal hearts valves distances with both MRI and autopsy.

Second, the inlet/outlet distance ratio was systematically  $\geq 0.9$  in normal hearts,  $\leq 0.81$  in LIAVV hearts and  $< 0.50$  in the AVSD heart. However, despite apparent differences between LIAVV/normal hearts, no statistically significant difference was found between inlet/outlet distance ratios. The measurement of the inlet/outlet distance ratio was performed in our study in adjunct to the usual measurement of the distance between the insertions of medial leaflets of atrioventricular valves. This evaluation is based on the assumption that all hearts with AVSD of any anatomic type have LIAVV. In addition, they share common anatomic characteristics such as an unwedged position of the aortic valve, a disparity between the inflow and outflow dimensions of the left ventricle caused by the deficiency of the postero-inferior part of the interventricular septum, and a trifoliate left atrioventricular valve [14]. By contrast, in isolated LIAVV as described by Fredouille et al. [2], the left atrioventricular valve has two and not three leaflets, appearing similar to a normal mitral valve. The only possible anatomic explanation to the lack of normal offsetting of the two atrioventricular valves in this case would thus be an abnormal insertion of the mitral valve of the ventricular septum, lower than usual. One hypothesis is that this lower insertion of the

mitral valve might be due to a deficiency of the postero-inferior part of the ventricular septum, similar to the defect present in AVSD, with complete attachment of the left atrioventricular valve on the crest of the ventricular septum but with a fusion of the two bridging leaflets leading to a complete anterior leaflet of the mitral valve. This feature can be appreciated by looking for a losangic membranous septum on macroscopy evaluation, present in the normal heart but absent in hearts with AVSD [15]. The presence of such a defect within the inlet portion of the ventricular septum can be appreciated on macroscopy evaluation by calculating the inlet/outlet ratio in a left-ventricular long-axis view. In the normal heart, this ratio is approximately 1:1. In complete AVSD, this ratio is lower, usually 1:2, depending of the size of the defect in the postero-inferior part of the ventricular septum. In LIAVV, this ratio is comprised between 1:1 and 1:2.

Ultimately, a step-wise process to a less-invasive examination of the fetal heart could be considered, which is noteworthy in a context of global decline of conventional autopsy and rise of minimally invasive autopsy [16]. Once LIAVV is confirmed on postmortem MRI, conventional autopsy can be performed, and the left ventricle can be opened to search for a postero-inferior deficiency of the ventricular septum closed by the attachments of the mitral valve. This deficiency confirmed in a significant number of cases would endorse the hypothesis that LIAVV could indeed be considered the most minor form of the AVSD spectrum. It would also validate the Fredouille et al. assumption that LIAVV may be a hallmark of Down syndrome and that LIAVV should be systematically searched for on prenatal echocardiography.

This study has several limitations. First, this is only a feasibility study performed on a very small sample of heart specimens. Obtaining high-spatial resolution with enough signal and contrast between tissues required very long acquisition times for 3D isotropic images (nearly 10 hours). These long acquisition times explain for the small number of specimens explored, resulting in limited statistical power. Second, our study focused only on fetuses in second and third trimesters, whereas most discrepancies between post-mortem cardiac MRI and fetopathology examination have been described for fetuses before 24 weeks of gestation [13]. Moreover, classifying a fetus with Down syndrome without apparent structural heart defects in the normal group is debatable. It has been recently reported that Down syndrome hearts without AVSD may have larger membranous septum, shorter ventricular septum and dysplasia of atrioventricular valves [17]. Nevertheless, none of these anomalies was observed on postmortem examination in this heart. Using high-field MRI systems is time-consuming, requires a certain degree of expertise to obtain and analyze the anatomical images and constitutes an expensive research imaging tool [18]. Nevertheless postmortem MRI is less costly than autopsy [19]. Moreover, high-field MRI is the most reliable postmortem imaging method for investigating the fetal heart [7,20]. Finally, larger prospective studies are needed to assess intra- and inter-observer reproducibility of measurements of LIAVV.

In conclusion, postmortem cardiac MRI performed at 4.7 Tesla could be a useful adjunct to pathologic examination in fetuses with abnormal insertion of atrioventricular valves seen on prenatal echocardiography and does not

require preparation of the fetal heart. This preliminary study could lead to a larger-scale prospective study of fetal heart specimens obtained after termination of pregnancy for aneuploidy, with a prenatal echocardiography showing abnormal insertion of atrioventricular valves.

## Human and animal rights

The authors declare that the work described has been carried out in accordance with the Declaration of Helsinki of the World Medical Association revised in 2013 for experiments involving humans as well as in accordance with the EU Directive 2010/63/EU for animal experiments.

## Informed consent and patient details

The authors declare that this report does not contain any personal information that could lead to the identification of the patient(s).

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## Contribution

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship.

## Disclosure of interest

The authors declare that they have no competing interest.

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