

## CARDIOMYOPATHY

# The Histological Basis of Late Gadolinium Enhancement Cardiovascular Magnetic Resonance in a Patient with Anderson-Fabry Disease

James C. Moon, MRCP,<sup>1</sup> Mary Sheppard, FRCPath,<sup>2</sup> Emma Reed, MB, BCh,<sup>2</sup> Phillip Lee, DM, FRCP,<sup>3</sup> Perry M. Elliott, MD, MRCP,<sup>4</sup> and Dudley J. Pennell, MD, FRCP<sup>1</sup>

CMR Unit, Royal Brompton Hospital, London, United Kingdom,<sup>1</sup> Department of Histopathology, Royal Brompton Hospital, London, United Kingdom,<sup>2</sup> National Hospital for Neurology and Neurosurgery, London, United Kingdom,<sup>3</sup> The Heart Hospital, London, United Kingdom<sup>4</sup>

## ABSTRACT

**Anderson-Fabry Disease (AFD) is a storage disease that mimics hypertrophic cardiomyopathy. Late gadolinium enhancement (LGE) by cardiovascular magnetic resonance occurs in approximately 50% of patients in the basal inferolateral LV wall, but how an intracellular storage disease causes focal LGE is unknown. We present a whole-heart histological validation that LGE is caused by focal myocardial collagen scarring. This scarring may be the substrate for electrical re-entry and sudden arrhythmic death. The reasons for this distribution of fibrosis are unclear, but may reflect inhomogeneous left ventricular wall stress.**

## INTRODUCTION

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder caused by mutations in the gene encoding for  $\alpha$ -galactosidase A. Reduced enzyme activity results in the intracellular accumulation of sphingolipid affecting many organs. In the heart, sphingolipid deposition causes myocardial hypertrophy, valvular dysfunction and cardiac arrhythmia. Up to 3% of males with otherwise unexplained LVH and 6% with late onset HCM have AFD (1, 2). Disease expression is also found in female heterozygotes (3). These abnormalities may be associated with sudden death and heart failure (4, 5).

Patients with familial cardiomyopathy have regions of myocardial late gadolinium enhancement (LGE) by cardiovascular magnetic resonance (CMR) (6–8). In HCM, LGE has been shown to represent myocardial fibrosis (9), and the extent is linked to markers of adverse clinical outcome (10). In AFD, LGE is found in up to 50% of patients, (11), but in a unique pattern affecting the basal inferolateral wall. It has been proposed that LGE in AFD may also represent focal myocardial fibrosis (11), which could be the substrate for heart failure and sudden death. However, as AFD is characterized by intracellular lipid accumulation, this hypothesis has not been verified. No histological correlations have been performed, and the predilection for the basal infero-lateral wall is unexplained (12).

We present the autopsy results from a male AFD patient, who was included in the original CMR report of LGE in the basal inferolateral wall (11), who subsequently sustained a sudden cardiac death.

## METHODS

In September 2002, a 57-year-old man with the classic features of AFD underwent CMR as part of a research study (11). He had been diagnosed with AFD on the basis of clinical presentation and a plasma  $\alpha$ -galactosidase A ( $\alpha$ -Gal) activity of  $<1.0$  nmol/hr/mL (normal range 4.0–21.9 nmol/hr/mL). He had received enzyme replacement therapy over 3 years. CMR showed massive symmetrical LVH with an LV mass index of  $243$  g/m<sup>2</sup> (normal  $<113$  g/m<sup>2</sup>). The maximum LV wall thickness

Received 18 September; accepted 18 December 2005

Keywords: Cardiomyopathy, Hypertrophy, Magnetic Resonance Imaging, Anderson-Fabry Disease.

Dr James Moon (Junior Research Fellowship) and the CMR Unit (Cardiovascular initiative grant) were supported by the British Heart Foundation. Support was also received from CORDA and Siemens Medical Systems.

Correspondence to:

Dr. James Moon

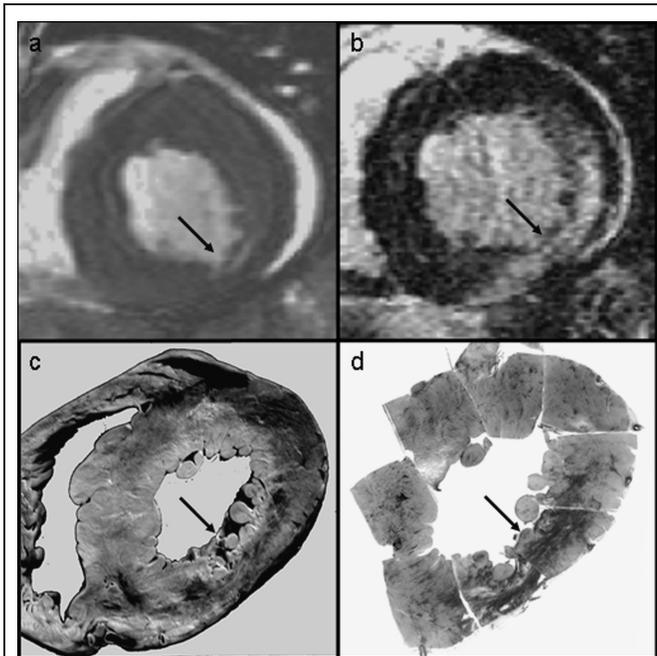
Royal Brompton Hospital

Sydney Street

London SW3 6NP

UK

email: James@moonmail.co.uk



**Figure 1.** Comparison of (A) in vivo diastolic cine CMR, (B) in vivo LGE CMR, (C) unstained gross specimen and (D) histologic sections stained with sirius red. All images are to the same scale. The heart has contracted down with fixation. There is basal inferolateral wall thinning associated with LGE, which is shown to represent a region of dense collagen (arrows).

was 27 mm. There was a focal region of thinning and akinesis of the basal infero-lateral of the LV (Fig. 1). Myocardial LGE was present at this location amounting to 35 g, 7% of total LV mass. After 22 months, during which he was stable clinically, he sustained a witnessed sudden cardiac death. An autopsy was performed and permission given by the relatives to perform a comprehensive examination of cardiac pathology.

### CMR

CMR was performed as previously described (10, 11). Briefly, imaging was performed on a 1.5T scanner (Siemens Sonata, Erlangen, Germany). Steady state free precession (SSFP) cines were acquired in the 2 chamber, 4 chamber and multiple contiguous short axis planes (7 mm slice thickness, 3 mm gap). A peripheral bolus injection of gadolinium-DTPA (0.1 mmol/kg) was then given and LGE acquired, starting at 5 minutes, using a segmented inversion-recovery sequence in the same short axis plane (13).

### Specimen preparation

Specimen preparation was as previously described (11). Briefly, routine fixation was performed with 10% formol saline and myocardial slices equivalent to the first and third basal in-vivo CMR slices cut. After photography, each slice was divided into 8 contiguous LV tissue blocks and stained with Sirius red (picro-sirius red) which stains collagen red and myocytes yellow (14, 15). The amount of collagen in each slice was quantified,

the slides were digitally scanned and reorganized to recreate the short axis slices for direct comparison with the CMR images.

### Data analysis

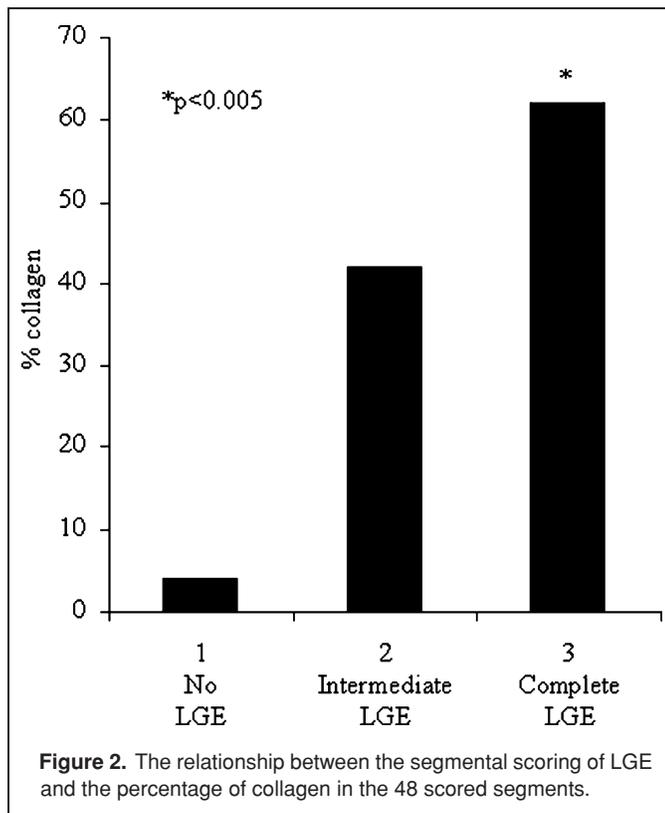
Analysis was performed as previously described (11), with the histology and CMR blinded and independent. Each of the 16 Sirius red stained blocks were divided into subendocardial, mesocardial and subepicardial layers. The area of Sirius red staining in each segment was quantified using manual planimetry with area quantification and expressed as a percentage of total myocardium in that segment. LGE was analyzed by visual assessment by 2 observers using a 3 point scale (LGE absent, intermediate or present) in a total of 48 segments. The results of the 2 observers were combined and LGE was described as present, absent or intermediate. A non-parametric version of the ANOVA test (Kruskal-Wallis) was used to compare the percentage of collagen between the 3 groups, and Wilcoxon rank test was used to compare between 2 groups.

## RESULTS

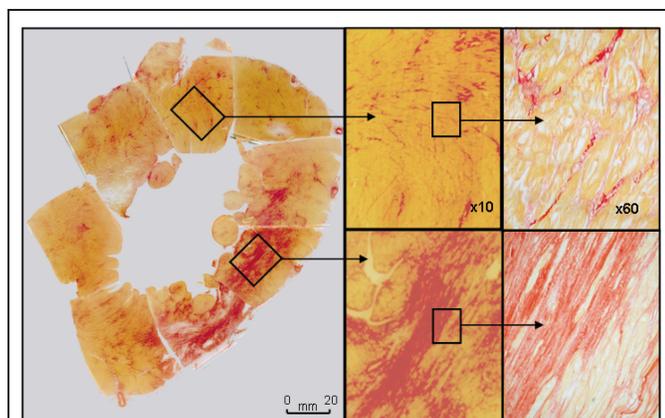
In Fig. 1, the most basal section by cine and LGE CMR are shown adjacent to the ex-vivo macroscopic section and reconstructed stained histological samples. There is clear concordance between the region of thinning and the location of LGE and collagen. There was extensive myocyte vacuolation throughout the myocardium. There was no evidence of epicardial coronary artery atherosclerosis, either in the segment of wall thinning or elsewhere. There was no evidence of extracellular lipid deposition. Overall, 18% of the examined heart (2 basal slices) was collagen, the extent varying from 0% (no detectable collagen at low power) to 100% (no detectable myocytes at low power) per segment. Segments with LGE were 62% collagen, segments with no LGE, 4% ( $p < 0.0004$ ), and intermediate segments had 42% collagen (Fig. 2). Although some segments were completely replaced by collagen, the pattern of collagen was ill defined and more loose than that found in myocardial infarction (Fig. 3).

## DISCUSSION

This case demonstrates that myocardial LGE in AFD is caused by myocardial collagen deposition. This is an important histological confirmation of the cause of LGE in AFD because of the rarity of such patients coming to autopsy who have had CMR. The results concur with a previous histological study in HCM, where LGE CMR was shown to occur in areas of fibrosis (9). Of significant interest is the possible relation between the finding of myocardial LGE and sudden death, as occurred in this patient. A link between the extent of LGE and risk factors for sudden death has been demonstrated in HCM, and it has been hypothesized that this results from macro re-entrant electrical circuits caused by the fibrosis (10). It has also been hypothesized that a link might be present between arrhythmia and the mid-wall longitudinal fiber fibrosis of dilated cardiomyopathy (7). Such hypotheses require prospective testing. In the context



of AFD, however, we have found LGE in 50% of patients, with what would normally be classified as early and advanced cardiac AFD in females (heterozygotes). Despite the limited data, the clinical course of this individual and a previous study of 4 patients with AFD who died of heart failure with aneurysms in the inferolateral wall (5) leads us to believe that the presence of LGE may be an adverse finding in AFD patients.



**Figure 3.** The histology of cardiac AFD. The basal inferior wall (lower middle and right panels) shows dense Sirius red staining on low power (middle), which on high power (right) is confirmed as collagen scarring (quantification, 45%). A remote region (upper middle and right panels) shows minimal collagen (quantification, 4%), but myocyte vacuolation from sphingolipid is present.

Non-ischemic LGE may be more difficult to assess and quantify than ischemic LGE, as can be seen by the number of ‘intermediate’ LGE segments scored in this paper. This reflects several processes. First, in myocardial infarction caused by epicardial coronary artery disease, the biology of the ischemic wavefront spreading from endo- to epicardium makes myocyte death within a voxel an essentially ‘all or none’ phenomenon, unless there is partial voluming. This makes interstitial volume differences large and dichotomous, unlike non-ischemic disease where interstitial expansion may be a more continuous variable. Secondly, when scoring LGE in myocardial infarction, the territorial and sub-endocardial involvement is helpful, whereas LGE in non-ischaemic disease may not demonstrate such specific distributions, although some are described (eg, LGE at the RV insertion points in sarcomeric HCM). For this reason, our practice is to take all views at least twice, usually with a phase swap. Finally, there may be variable ‘background’ interstitial expansion, particular with differential hypertrophy, which may make nulling variable across the myocardium, seen for example in long axis views of apical HCM. Other techniques such as T1 mapping may be helpful.

This study gives few clues as to why fibrosis has a peculiar predilection for the inferolateral wall. There was no evidence of increased lipid accumulation in the area, and the feeding arteries and arterioles were normal. There is rather little parallel from other cardiological conditions from which to draw assistance. It has recently been shown using CMR that myocarditis has a predilection for the lateral and inferolateral regions, with abnormality focused in the epicardium and mid-wall (16). It is possible that cardiac involvement in AFD might predispose the heart to sub-clinical myocarditis, leading to injury. Another possibility for the location of the fibrosis might be that the cardiac involvement could impair resistance to physical stress within the myocardium, which might be most prominent in this region where significant shear forces combine with a watershed vascular territory. Possible supporting evidence for this is the lateral wall involvement which occurs in Chagas cardiomyopathy (17), the autonomic denervation in left ventricular involvement in arrhythmogenic right ventricular cardiomyopathy (18), and the common location of granulomas and aneurysms in the lateral wall in sarcoidosis (19). Although the link between intracellular lipid accumulation and focal interstitial collagen scarring is unknown, the fact that myocardial fibrosis occurs in AFD, suggests that there might be a rationale for early enzyme replacement therapy in order to prevent myocardial fibrosis. Such a hypothesis requires prospective testing.

## REFERENCES

1. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med* 1995;333:288–93.
2. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, Elliott PM. Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. *Circulation* 2002;105:1407–11.

3. Chimenti C, Pieroni M, Morgante E, Antuzzi D, Russo A, Russo MA, Maseri A, Frustaci A. Prevalence of Fabry Disease in Female Patients With Late-Onset Hypertrophic Cardiomyopathy. *Circulation* 2004;110:1047–53.
4. Desnick RJ, Ioannou YA, Eng CM.  $\alpha$ -Galactosidase A deficiency: Fabry disease. In: Scriver CR (ed). *The metabolic and molecular basis of inherited disease*. New York, McGraw-Hill, 2001, pp 3733–74.
5. Teraguchi H, Takenaka T, Yoshida A, Taguchi S, Ninomiya K, Yoshida H, Horinouchi M, Yonezawa S, Nakao S, Minagoe S, Tei S. End-stage cardiac manifestations and autopsy findings in patients with cardiac Fabry disease. *J Cardiol* 2004;43:98–9.
6. Choudhury L, Mahrholdt H, Wagner A, Choi KM, Elliott MD, Klocke FJ, Bonow RO, Judd RM, Kim RJ. Myocardial scarring in asymptomatic or mildly symptomatic patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2002;40:2156–64.
7. McCrohon JA, Moon JC, Prasad SK, McKenna WJ, Lorenz CH, Coats AJ, Pennell DJ. Differentiation of heart failure related to dilated cardiomyopathy and coronary artery disease using gadolinium-enhanced cardiovascular magnetic resonance. *Circulation* 2003;108:54–9.
8. Moon JC, Mund y HR, Lee PJ, Mohiaddin RH, Pennell DJ. Images in cardiovascular medicine. Myocardial fibrosis in glycogen storage disease type III. *Circulation* 2003;107:e47.
9. Moon JC, Reed E, Sheppard M, Elkington AG, Ho SY, Burke M, Petrou M, Pennell DJ. The Histological Basis of Myocardial Hypereenhancement by Gadolinium Cardiovascular Magnetic Resonance in Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 2004;43:2260–4.
10. Moon JCC, McKenna W, McCrohon JA, Elliott PM, Smith GS, Pennell DJ. Towards clinical risk assessment in hypertrophic cardiomyopathy with gadolinium cardiovascular magnetic resonance. *J Am Coll Cardiol* 2003;41:1561–7.
11. Moon JC, Sachdev B, Elkington AG, McKenna WJ, Mehta A, Pennell DJ, Elliott PM. Gadolinium Enhanced Cardiovascular Magnetic Resonance in Anderson-Fabry Disease: Evidence for a Disease specific Abnormality of the Myocardial Interstitium. *Eur Heart J* 2003;24:2151–5.
12. Pennell DJ, Sechtem U, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, van Rossum AC, Shaw LJ, Yucel EK. Clinical indications for cardiovascular magnetic resonance: Consensus Panel report. *Eur Heart J* 2004;6:1–26.
13. Simonetti OP, Kim RJ, Fieno DS, Hillenbrand HB, Wu E, Bundy JM, Finn JP, Judd RM. An improved MR imaging technique for the visualization of myocardial infarction. *Radiology* 2001;218:215–23.
14. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979;11:447–55.
15. Puchtler H, Waldrop FS, Valentine LS. Polarization microscopic studies of connective tissue stained with picro-sirius red FBA. *Beitr Pathol* 1973;150:174–87.
16. Mahrholdt H, Goedecke C, Wagner A, Meinhardt G, Athanasiadis A, Vogelsberg H, Fritz P, Klingel K, Kandolf R, Sechtem U. Cardiovascular magnetic resonance assessment of human myocarditis: a comparison to histology and molecular pathology. *Circulation* 2004;109:1250–8.
17. Rochitte CE, Oliveria PF, Andrade JM. Chagas Disease Is Characterized by Specific Pattern and Location of Myocardial Delayed Enhanced MRI. *J Cardiovasc Magn Reson* 2003;5:13–14.
18. Wichter T, Hindricks G, Lerch H, et al. Regional myocardial sympathetic dysinnervation in arrhythmogenic right ventricular cardiomyopathy. An analysis using 123I-meta-iodobenzylguanidine scintigraphy. *Circulation* 1994;89:667–83.
19. Roberts WC, McAllister HA, Ferrans VJ. Sarcoidosis of the heart. A clinicopathologic study of 35 necropsy patients (group I) and review of 78 previously described necropsy patients (group II). *Am J Med* 1977;63:86–108.