Prospective Cardiovascular Magnetic Resonance Imaging in Adults with Alström Syndrome: Silent Progression of Diffuse Interstitial Fibrosis.

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Research

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Abstract

Background Alström syndrome (ALMS) is a rare ciliopathy characterised by early onset insulin resistance, obesity, and dyslipidaemia and is a model for diseases that have huge social, health and economic impact. Cardiomyopathy develops in the majority, with high rates of morbidity and mortality, the definitive feature of which is the presence of coarse replacement fibrosis and diffuse myocardial fibrosis (DIF). The pathogenesis of heart failure is thought to involve fibroblast accumulation and expansion of the extracellular matrix with excess protein deposition, leading to distorted organ architecture and impaired contractile function. Consecutive adults with genetically proven ALMS attending the National Centre for Rare Disease in Birmingham, England were studied. All patients underwent serial CMR, echocardiography and venous blood sampling following standard clinical practice, with computed tomography coronary angiography (CTCA) performed to assess severity of CAD. The aims of this study were: 1) to evaluate changes over time in DIF by cardiovascular magnetic resonance tissue characterization in ALMS; 2) to examine whether changes in DIF are associated with alteration in systolic or diastolic function; and 3) to evaluate the frequency and severity of coronary artery disease as a confounder for progression of ischaemic versus non-ischaemic fibrosis. Results In total, 30/32 adults (63% male; 67% White British) participated. The median age at first scan was 21.3 years (interquartile range: 19.0–32.6) and participants were followed for a maximum of 67 months. Only 4 patients had significant coronary artery stenosis on post-mortem, invasive or CTCA. Mid short axis myocardial T1 times, myocardial extracellular volume, and left ventricular mass increased significantly over time, by an average of 21.8ms (95% CI 17.4–26.1; p<0.001), 0.011 (0.006–0.016, p<0.001), and 2.8 g/m² (1.9–3.7; p<0.001) per year, respectively. These changes were not associated with significant deterioration in myocardial structure or function. Conclusions This is the first comprehensive prospective study demonstrating progression of DIF in ALMS over time, although no structural or functional consequences were noted within a median three and a half years’ follow up. Further study is warranted to determine whether DIF is a bystander or the driver to impaired contractile function, heart failure and death.

Introduction

Alström syndrome (ALMS) is a rare autosomal recessive cardiomyopathy (OMIM 203800) characterised by multiorgan disease. Infantile cardiomyopathy and retinal cone rod dystrophy are the earliest and most frequent manifestations of the syndrome. The most severe and often lethal complication of ALMS is heart failure due to cardiomyopathy.¹ Cardiomyopathy affects up to 65% of adolescents and adults, with high rates of morbidity and mortality.² Both the quality and length of life are reduced in adults with ALMS, and few survive beyond the age of 50. Autopsy data has demonstrated replacement myocardial fibrosis in non-coronary artery patterns, and diffuse interstitial fibrosis (DIF) has been detected on cardiovascular magnetic resonance (CMR) by elevation in T1 relaxation times and increased extracellular volume (ECV) in cross-sectional studies of ALMS.⁴ These changes were associated with sub-clinical impairment of left ventricular (LV) function, assessed through change in myocardial strain. In many cardiovascular diseases, it has been postulated that the development of DIF over time may be a driver of abnormal myocardial deformation and diastolic dysfunction, leading ultimately to systolic heart failure.⁵ While ALMS is rare, it offers a model of myocardial disease in which disease progression may be rapid, although serial assessment has not previously been performed.

Given the frequency of obesity, insulin resistance and metabolic syndrome in ALMS, ischaemic heart disease may well represent a potential confounder to increased T1 relaxation times and ECV.⁶ Despite the high burden of CV risk factors, reported cases of coronary artery disease (CAD) are rare and data has been limited.⁷ Moreover, defining serial changes in T1 and ECV relative to the frequency of coronary disease is important on a clinical basis, since primary and secondary treatment strategies differ. Therefore, the aims of this study were: 1) to evaluate longitudinal changes in DIF by CMR tissue characterization in adults with ALMS; 2) to examine whether changes in DIF could be associated with alteration in systolic or diastolic function; and 3) to evaluate the frequency and severity of coronary artery disease as a confounder for progression of ischaemic versus non-ischaemic fibrosis.

Methods

Study Design

Between March 2012 and January 2018, 32 consecutive adults with genetically proven ALMS attending the National Centre for Alstrom syndrome at the Queen Elizabeth Hospital Birmingham, United Kingdom (UK) were evaluated as part of standard clinical care. The clinic is unusual in that it is patient-centred, run in association with the charity Alström Syndrome UK and is multidisciplinary (MDT). Patients attend for two days and in this time, undergo all their relevant investigations, and are seen by all members of the MDT. At the first and subsequent visit, patients underwent annual disease monitoring following a standardized clinical protocol, including: biochemistry; echocardiography; and CMR. This was an observational, retrospective audit, utilizing existing data collected as part of clinical service. It was approved by local clinical governance committees (CARMS-15674) and conformed to the principles of Good Clinical Practice guidelines. Patients were eligible for inclusion in this audit if they were ≥ 16 years of age; had a genetically confirmed diagnosis of ALMS; and had been followed up in the service for a minimum of one year. Patients were excluded if they had an absolute contra-indication to CMR (1 patient with implantable cardio defibrillator) or a relative contra-indication for the study (1 patient was on a research drug trial). Otherwise, all adult patients in the service were included. Serial
CMR and single computed tomography coronary angiography (CTCA) were included as standard for ALMS patients. Annual CMR was instituted from the start of the clinical service, while CTCA was introduced soon after the incidental finding of an occluded right coronary artery in an asymptomatic 38 year old male.

**Investigations**

**Blood markers:** Venous blood samples were collected for haematology, biochemistry, and lipid profiles. Serum N-terminal pro B natriuretic peptide (NT-proBNP; ng/L) was measured by sandwich immunoassay with magnetic particle separation and chemiluminescent detection on an E170 analyser (Roche Diagnostics, Burgess Hill, United Kingdom) with a lower limit of detection of 0.6 pmol/L. Dyslipidaemia was defined as fasting cholesterol >5 mmol/L and/or triglycerides >1.7 mmol/L.

**Echocardiography:** Resting transthoracic echocardiography (TTE; ie33, Phillips) was performed by an accredited sonographer according to the British Society of Echocardiography minimum dataset. Diastolic function was graded by an experienced cardiologist specialising in echocardiography (RPS) according to current guidelines.

**Cardiac magnetic resonance imaging:** Contrast enhanced CMR (1.5T Avanto, Siemens Healthcare, Erlangen, Germany) was performed in-line with standard protocols to obtain LV and right ventricular (RV) dimensions, volumes, and mass on a bi-annual basis. A steady-state free precession, single breath hold modified Look-Locker inversion recovery (MOLLI) sequence was used for T1 mapping in the basal and mid left ventricular short axis levels and the horizontal long-axis, before and 15-20 minutes after administration of gadolinium-based contrast agent (GBCA). Average scan parameters; 8 mm slice with a 192 read-out matrix, 6/8 phase partial Fourier with 81% phase resolution, field-of-view 320 × 320 mm², repetition time 2.4 ms, echo time 1.01 ms, 11 phases [3, 3, 5 scheme], total breath hold 17 R-R intervals. LGE imaging was performed 7-10 minutes after 0.15 mmol/kg of GBCA given as a bolus (Gadovist, Bayer Health Care) using phase sensitive inversion recovery. Analysis of volumes, mass, and function was performed off-line using Cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada) according to the Society for CMR guidelines for reporting, with parameters indexed to height and body surface area (BSA), where appropriate. Tissue tracking in the 3 long axis views was performed offline to assess 2D global longitudinal strain (GLS), according to previously published formulae. Analysis of T1 was performed with manual contouring to define a region of interest within the LV myocardium at basal and mid ventricular level (short-axis) on matched pre- and post-contrast images within the septum, as well as within the septum in the horizontal long axis view, and used to calculate myocardial extracellular volume (ECV) by validated formulae. T1 values were measured in ‘normal myocardium’ defined by the absence of LGE. In addition, both short axis maps (basal and mid) were also manually contoured for endo- and epicardial borders, and partial voluming of blood minimised by using a 20% offset from the endo- and epicardial border. Stability of T1 measurement over time was confirmed by weekly analysis of a phantom within the magnet. Analysis of all CMR parameters was performed by a single observer (SB) blinded to the identity of the subject, date, and time. A randomly selected subset of 10 patients were selected for intra- and inter-observer reproducibility studies by SB and RV. Bland-Altman plots were used to assess the reproducibility of these measurements, with mean differences calculated to identify any bias. Agreement was further quantified using intraclass correlation coefficients (ICCs), using two-way random effects models with absolute agreement.

**Phantom studies for T1 stability:** T1 times stability over time was assessed using a phantom made of high density plastic beads of agar/nickle chloride in plastic tubes. High-density polyethylene macrobeads encased these plastic tubes arranged in a 3 x 3 array, with resin layer at the base. Each tube represented a range of T1 times. These were scanned regularly for 12 months on a fortnightly basis using the T1 protocol described above. A tight shim was set with all images acquired at iso-center. The analysis was done using ROI as described above.

**Computed tomographic coronary angiography:** Participants without renal dysfunction had a single CT coronary artery calcium (CAC) score and CTCA performed on dual energy source 64 multi-slice scanner (Siemens Somaton Definition, Erlangen, Germany) using retrospective electrocardiogram (ECG) gated acquisition. A 20 mL test bolus of contrast was used to set delay and 80 mL of contrast given for final image acquisitions. Significant CAD was defined as a lesion >70% on post-mortem, invasive or CT coronary angiography.

**Statistical Methods**

The baseline demographics of the cohort were summarised, with continuous variables reported as means ± standard deviations (SDs) where normally distributed, and as medians and interquartile ranges (IQRs) otherwise. Changes over time in a range of markers were then assessed. Initially, Spearman’s correlation coefficients (rho) were produced to measure the correlation between each marker and the timing of the measurement, relative to each patient's first scan. However, this approach did not account for the non-independence of the repeated scans on each patient. As a result, the analysis was repeated using a generalized estimating equation (GEE) approach. A separate model was produced for each marker, with the timing of the measurement, relative to each patient's first scan as a continuous covariate, and the patient identification number (ID) as a nominal factor. The patient ID was set as the subject effect, with the within-subject effect being the timing of the scan, relative to the initial scan, rounded to the nearest 6 months. An AR (1) correlation structure was assumed throughout. Analyses were then performed to assess whether changes over time in T1 corresponded to changes in other markers. For each marker, separate linear regression models were
produced for each patient that had data with at least two scans, with the timing of the measurement relative to baseline, as a covariate. The gradients from the resulting models were then used to represent the rate of change in the markers and were compared using Spearman's rho correlation coefficients. In addition, comparisons of gradients across dichotomous variables (e.g. gender) were performed using independent samples t-tests. All analyses were performed using IBM SPSS 22 (IBM Corp. Armonk, NY), with p<0.05 deemed to be indicative of statistical significance throughout.

Results

Study Population

There are 38 known adult ALMS patients within the United Kingdom, of whom 32 regularly attend the specialized service in Birmingham. Of these, two were excluded due to contra-indications to CMR (1 implantable cardioverter-defibrillator; 1 patient within research study), hence the analysis was based on the remaining 30. Baseline demographics and imaging data are presented in Table 1 and genetic data in Table 2. Family history of premature CAD was not recorded. The median age at the first scan was 21.3 years (IQR: 19.0-32.6), 63% of patients were male and 67% were of White British ethnicity. The first CMR scan was performed the day before or same day as the first clinical attendance in the service. From first CMR, the median follow-up time was 45 months (maximum 67 months), during which time 76 additional scans were performed, a median of 3 follow-up scans per patient, with 4 patients only having a single scan during the follow-up period. More than half the cohort had conventional cardiovascular risk factors (Table 1). Six patients (20%) had elevated NT-proBNP (≥144 ng/L). At baseline, LV volumes, ejection fraction (EF) and mass were within normal limits for age, sex and body surface area on CMR. In total, 14 patients had evidence of LGE. Of these, 2 patients had LGE at the insertion of the RV into the septum (insertion point LGE), 1 had focal subendocardial and 2 had focal epicardial LGE. Mid wall LGE was present in 5 patients, while 4 patients had extensive LV and RV LGE. The LGE pattern did not correspond to a specific coronary artery disease distribution.

Serial Data

On correlation analysis, T1 basal and mid ventricular level (short-axis; both p<0.001), ECV basal and mid ventricular level (short-axis; p=0.081 and p=0.064), and LV mass (p=0.008) all increased over time. Analysis of serial measurements using a GEE approach found significant increases over time in T1, as measured on the basal and mid ventricular (short-axis) levels, with similar gradients of 25.8ms per year (95% CI: 20.0 – 31.7, p<0.001) and 21.8ms per year (95% CI: 17.4 – 26.1, p<0.001), respectively (Figure 1a). Significant increases in ECV were also observed, with gradients of 0.014 per year (95% CI: 0.008 – 0.020, p<0.001) and 0.011 per year (95% CI: 0.006 – 0.016, p<0.001) for basal and mid ventricular levels, respectively (Figure 1b). In addition, the LV mass index was found to increase significantly over time, by an average of 2.8 g/m² per year (95% CI: 1.9 - 3.7 g/m², p<0.001). Analyses using a correlation approach returned consistent findings (Table 3).

Two patients developed LGE during follow up, 1 of whom developed new RV insertion point LGE and 1 developed subendocardial LGE in the mid anteroseptum (participants 10 & 11, Supplementary Table 1). No significant changes over time were detected in LV end-diastolic volume indexed to BSA (LVEDVi, p=0.909), LV end-systolic volume indexed to BSA (LVESVi, p=0.067), LV ejection fraction (EF, p=0.575), left atrial volume indexed (LAVi, p=0.409), or LV GLS (GLS, p=0.809). In addition, no significant changes over time were noted in diastolic function on echocardiography, including mitral early filling (E)/atrial filling (A) ratio (p=0.313), and average E/early myocardial relaxation velocity (e', p=0.299). (Table 3)

Factors Associated with Change in ECV or T1

Changes in ECV and T1 were not found to be significantly associated with differences in LV volumes, mass, or EF over follow-up (Table 4). There was also no evidence of a significant association between the change in ECV or T1 and markers of diastolic function (E/A; E/e' average; LAV).

Coronary Artery Disease

Presence or absence of CAD could not be established in 3 of the 30 patients. One patient was not assessed, as they refused to undergo CAC/CTCA due to a combination of claustrophobia and needle phobia, while 2 patients had not undergone CAD assessment at time of data collection. Of the remaining 27 patients, 3 patients presented with symptoms and were diagnosed with CAD. Of these, one died before a CTA could be performed, but post-mortem showed a right coronary artery occluded with thrombus. The second patient had invasive coronary angiography following onset of symptoms and was found to have severe CAD. The third patient had CAD (>70% stenosis) identified on CTA, followed by percutaneous coronary intervention with stent implantation in the mid left anterior descending artery. The remaining 24 asymptomatic patients all had a CTA done, and 22 had CAC scoring. Of these, 4 were found to have elevated CAC Agatston scores of 48, 69, 156, and 209. These 4 patients were found to have mild atheroma with no flow-limiting stenosis, with CTA being normal in the remainder. In summary, a total of 3/27 (11%) had significant CAD defined by >70% stenosis, whilst a further 4/27 (15%) had non flow-limiting atheroma. Further details about these 7 patients are reported in Table 6.
Intra- and Inter-Observer Reproducibility, Phantom Study and Normal Ranges

Ten CMR studies were randomly selected for assessment of intra- and inter-observer variation. There was no consistent pattern of inter-observer bias on Bland-Altman analyses. Intra-observer mean bias for basal T1 was 5.8 ms (95% CI -4.3, 15.8 ms), with an ICC (random model) of 0.9 (p<0.001), whilst inter-observer mean bias for basal T1 was 6.4 ms (95% CI -7.2, 20 ms), with an ICC of (0.9, p<0.001). Normal T1 measurements and ECV values using the same protocol in normal volunteers on the same scanner were as follows: (n=26) mid SAX pre contrast T1 970 ± 11ms; post contrast T1 522 ± 58ms; ECV 0.25 ± 0.01. Over 12 months, 25 MOLLI datasets were collected. The scanner room temperature was stable at 20.96 ± 0.98 C. Coefficients of variation across the 9 tubes were stable and ranged from 0.436 to 0.872 % when unadjusted for temperature and improved to and 0.436 to 0.868 % with correction for room temperature.

Discussion

This is the first longitudinal study to demonstrate an increase in T1 and ECV over time, paralleled by an increase in LV mass in ALMS. Despite changes in T1 and ECV, there was no evidence of systolic or diastolic contractile dysfunction, either in terms of EF, GLS, E/A, E/e’, LA volume, or elevation in biomarker (NT-proBNP). The increased T1 and ECV were not significantly associated with the presence or absence of CAD. These data are consistent with progressive myocardial fibrosis over time in ALMS, although no structural or functional consequences were noted within a median three and a half years’ follow up. Why is this finding in the rare disease Alstrom of importance? ALMS is characterized by obesity, insulin resistance and dyslipidaemia, diseases that are common but are projected to increase beyond that expected by evolving population demographics. Furthermore, heart failure is highly prevalent in each, with adverse prognosis whether or not associated with coronary artery disease. Given the premature onset of cardiomyopathy, ALMS is a paradigm to investigate the role of DIF in pathogenesis of heart failure, and whether this may be a target for therapeutic intervention or simply a by-stander to other disease processes. Our data identifies progression of DIF that is independent of coronary artery disease, but further research is needed to clarify relationship with functional decline.

Previous studies have demonstrated that T1 values and ECV are increased in ALMS compared to control subjects. These data are consistent with post-mortem histology studies demonstrating both coarse and DIF in adults with ALMS. Although the data correlating high T1, increased ECV and histology in ALMS are limited, T1 mapping and ECV on CMR as markers of DIF have been validated histologically in other conditions. Longitudinal studies have shown T1 mapping to be a robust parameter, with little variability in healthy individuals over time, and in our study, the increase was measured in a single magnet using the same sequence with stable signal confirmed by phantom. The increase in T1 and ECV was not, however, associated with a change in systolic or diastolic contractility, measured either by CMR or echocardiography. In our previous cross-sectional study, an association was found between increased T1 and ECV and altered GLS measured using CMR tagging, and it may be that the method used in this serial study, tissue-tracking, was less sensitive. It is also notable that the size of the change was small and that change in function may require longer follow-up to have a significant impact. This is consistent with lack of functional change on serial CMR over 2 years seen in patients with chronic stable cardiomyopathy who had a detectable increase in T1 times. Further longitudinal studies are needed to see if the increased T1 and ECV are associated with the development of systolic dysfunction, heart failure and death, as has been demonstrated in other populations.

Infantile cardiomyopathy is a common finding in infants with ALMS, presenting within the first 3 months of life. The majority (74%) survive, with response to standard heart failure therapy resulting in recovery of cardiac function in the majority. Recent evidence suggests that the ALMS1 protein has a role in perinatal cardiomyocyte cell division and replication, and that its deficiency can cause mitogenic cardiomyopathy. The mechanisms leading to infantile cardiomyopathy appear to differ from those seen in adult cardiomyopathy, where myocardial fibrosis plays a dominant role. In keeping with this, there was no detectable difference in structure, function, or progression of T1/ECV between those patients with and those without a history of infantile disease. In addition, given the risk profile of the ALMS cohort under care, it was thought important to investigate the potential impact of CAD on the development of myocardial fibrosis and heart failure. More than a quarter of the cohort (7/27) had evidence of coronary atheroma, although only 3 patients had flow-limiting disease, 2 of whom were symptomatic. The prevalence of CAD was not found to influence the progression of T1 or ECV significantly in this study, although the statistical power of this analysis was low due to small numbers.

Limitations. The primary limitation of this study, inevitably, is the relatively small sample size. As a result of this, the statistical power of analyses is low, meaning that only relatively large effects would have been detectable, increasing the risk of false negatives. This was especially applicable to the analyses comparing the T1 and ECV gradients across baseline factors, particularly for comparisons across groups with unequal numbers of patients, such as the presence of CAD (N=3 vs. N=20). Although numbers were small, participants had a genetically confirmed rare disease, recruited from a national centre with one of the largest adult cohorts available. Where significant trends over time were detected, the rates of change were generally small, and may not have been sufficient to have clinical impact in the short-term. However, the fact that the trajectories were similar across the whole cohort, and that testing of the scanner using a phantom within the magnet used demonstrated marked stability of the signal over time, would suggest that these trends are reflective of genuine progression of fibrosis.
Baseline T1 values were heterogeneous in our cohort, consistent with our previous and other research in ALMS, although this may in part be accounted for differences in age, gender and co-existing metabolic status. This significant inter-individual variation suggests that development of fibrosis is not an inevitable consequence of the genetic defect alone and that environmental factors may also play an important role. We acknowledge however, the possibility of myocardial fat deposition or pseudo normalization of T1 due to the combined presence of lipid and fibrosis within the LV. Our own limited post-mortem data did not identify significant myocardial fat deposition in ALMS but recognise that further tissue characterisation, including T2 mapping, would have been enlightening.

**Conclusion**

This is the first comprehensive longitudinal study to suggest that myocardial fibrosis in ALMS progresses over time. There were no associated functional changes in systolic or diastolic function over follow-up, suggesting that these occur later in the natural history of ALMS cardiomyopathy. Longer term studies should be performed to confirm whether the development of DIF over time may be the primary driver rather than a consequence of ventricular dysfunction and heart failure.

**Abbreviations**

| Abbreviation | Description                          |
|--------------|--------------------------------------|
| A            | Mitral atrial filling velocity       |
| ALMS         | Alstrom syndrome                     |
| BSA          | Body surface area                    |
| CAC          | Coronary artery calcium              |
| CAD          | Coronary artery disease              |
| CMR          | Cardiovascular magnetic resonance imaging |
| CTCA         | Computed Tomography Coronary Angiography |
| DIF          | Diffuse interstitial fibrosis        |
| E            | Mitral early filling velocity        |
| e’           | Early myocardial relaxation velocity |
| ECG          | Electrocardiogram                    |
| ECV          | Extracellular volume                 |
| EF           | Ejection fraction                    |
| GBCA         | Gadolinium-based contrast agent      |
| GEE          | Generalised estimating equation      |
| GLS          | Global longitudinal strain           |
| ICC          | Intraclass correlation coefficient    |
| ID           | Identification number                |
| IQR          | Interquartile range                  |
| LAV          | Left atrial volume                   |
| LGE          | Late gadolinium enhancement          |
| LV           | Left ventricle                       |
| EDV          | End diastolic volume                 |
| EDVi         | End diastolic volume indexed to body surface area |
ESV End systolic volume
ESVi End systolic volume indexed to body surface area
MOLLI Modified look-locker inversion recovery sequence
NT pro-BNP N-terminal pro B natriuretic peptide
RV Right ventricle
SD Standard deviation
TTE Transthoracic echocardiography

Declarations

Ethics approval and consent to participate: This study was approved by local clinical governance committees and conformed to the principles of Good Clinical Practice. This study utilized clinical data collected in the course of normal patient care (without intention to use it for research at time of collection) and thus does not require ethical approval, consistent with Health Regulation Authority guidance.

Consent for publication: Not applicable.

Availability of data and material: The datasets generated and/or analysed during the current study are not publicly available as the single centre location of the study, combined with the nature of the disease, mean that individual patients might be identified through the comprehensive data and material used in this study. These are available from the corresponding author on reasonable request, subject to discussion on patient identifiable information.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: RS, TH and NCE conceived the study, reviewed data and contributed to the manuscript; SB, RD, RV and BL collated data, contributed to analysis and to the manuscript; JH provided statistical expertise and analysis; LF reviewed and critically contributed to the manuscript. All authors read and approved the final manuscript

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Tables

Table 1. Baseline Patient Demographic and Cardiovascular Imaging Data
| Factor               | N   | Statistic       |
|---------------------|-----|-----------------|
| **Demographics**    |     |                 |
| Age at first scan   | 30  | 21 (19 - 33)    |
| (years)             |     |                 |
| Male Gender (%)     | 30  | 19 (63%)        |
| White British (%)   | 30  | 20 (67%)        |
| BMI (kg/m$^2$)      | 30  | 29 (26 - 32)    |
| Systolic BP (mmHg)  | 30  | 130 ± 19        |
| Diastolic BP (mmHg) | 30  | 82 ± 12         |
| Heart rate (beats/min) | 30  | 86 ± 17        |
| **CMR**             |     |                 |
| T1 Basal (ms)       | 30  | 937 (913-1030)  |
| T1 SAX Basal ECV    | 30  | 0.27 (0.22-0.33)|
| LVEDVi (mL/m$^2$)   | 30  | 58 ± 12         |
| LVEF (%)            | 30  | 64 ± 9          |
| LV mass index (g/m$^2$) | 30  | 55 (51 - 61)   |
| LAV index (mL/m$^2$) | 30  | 34 (25 - 43)   |
| GLS (%)             | 30  | 16 ± 3          |
| **Echocardiogram**  |     |                 |
| E/A ratio           | 29  | 1.5 (1.3 - 1.6) |
| Average E/e’ ratio  | 30  | 7.4 ± 2.1       |
| e’ ave (cm/s)       | 27  | 9.1 (7.7 - 11.0)|
| **Blood markers**   |     |                 |
| Haemoglobin (g/L)   | 30  | 140 ± 23        |
| HbA1c (mmol/mol)    | 29  | 49 (39 - 71)    |
| C-peptide (pmol/L)  | 30  | 3136 (1516 - 4113)|
| Cholesterol (mmol/L)| 30  | 4.8 ± 1.3       |
| Triglycerides (mmol/L) | 30  | 2.5 (1.7 - 4.2) |
| NT-proBNP (ng/L) | 30 | 51 (25 - 93) |
|------------------|----|-------------|
| eGFR (mL/min/1.73 m²) | 30 | 108 (62 - 123) |

**Comorbidities**

- Hypertension 30 17 (57%)
- Dyslipidaemia 30 18 (60%)
- Infantile cardiomyopathy 30 12 (40%)
- Diabetes 30 18 (60%)
- CKD 30 18 (60%)

BMI indicates body mass index; BP, blood pressure; CMR, cardiovascular magnetic resonance; E/A ratio, mitral early filling (E)/atrial filling (A) ratio; E/e’, mitral early filling (E)/early myocardial relaxation velocity (e’); ECV, extracellular volume; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; HbA1c, glycated haemoglobin A1c; IQR = interquartile range; LAV, left atrial volume; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume indexed; NT-proBNP, serum N-terminal pro B natriuretic peptide; RV, right ventricle; SAX, short axis; CKD chronic kidney disease.

Data are reported as N (%), mean±SD, or median (IQR), as applicable.

Table 2: Mutations in ALMS gene in study participants.
| Mutation 1 | Exome 1 | Allele 1 nucleotide change | Allele_1 amino acid change | Homo/ Hetero/CH | Mutation 2 | Exome 2 | Allele 2 nucleotide change | Allele_2 amino acid change |
|-----------|---------|---------------------------|---------------------------|-----------------|-----------|---------|---------------------------|---------------------------|
| Nonsense  | 8       | c.2041C>T                 | p.Arg681X                 | Homo            | ?Nonsense | 8       | c.2041C>T                 | p.Arg681X                 |
| Nonsense  | 8       | c.6823C>T                 | p.Arg2275X                | CH              | Nonsense  | 10      | c.9535C>T                 | p.Arg3179X                |
| Nonsense  | 8       | c.2822T>A                 | p.Leu941X                 | CH              | Frameshift| 16      | c.10775delC               | p.Thr3592Lysfs*6          |
| Frameshift| 8       | c.6584delA                | p.Lys2195Serfx10          | CH              | Nonsense  | 5       | c.1008_1009delTG          | p.Cys336fsX1             |
| Nonsense  | 10      | c.8002C>T                 | p.Arg2668X                | CH              | Nonsense  | 16      | c.10879C>T                | p.Arg3627x                |
| Nonsense  | 10      | c.9001C>T                 | p.Gln3081X                | Hetero          |           |         |                           |                           |
| Frameshift| 8       | c.6895delG                | p.val2299TrpfsX43         | CH              | Frameshift| 16      | c.11443C>T                | p.Gln3815X                |
| Nonsense  | 16      | 11107C>T                  | p.Arg3703X                | Hetero          |           |         |                           |                           |
| Nonsense  | 16      | 11107C>T                  | p.Arg3703X                | Homo            | Nonsense  | 16      | 11107C>T                  | p.Arg3703X                |
| Frameshift| 16      | c.10579_1580delAT         | p.Met3527Valfs*13         | CH              | Frameshift| 18      | c.11856delC               | p.Asn3952Lysfs*41         |
| Frameshift| 16      | c.10769delC               | p.Thr3590LysfsX6          | CH              | Missense  | 8       | c.5356A>G                 | p.Asn1786Asp             |
| Nonsense  | 16      | c.11107C>T                | p.Arg3703                 | Hetero          |           |         |                           |                           |
| Nonsense  | 8       | c.6823C>T                 | p.Arg2275X                | CH              | Nonsense  | 16      | c.10477C>T                | p.Gln3493X                |
| Frameshift| 8       | c.1729delA                | p.Gly577Glyfsx17          | CH              | Nonsense  | 16      | c.10477C>T                | p.Gln3493X                |
| 8         |         | c.6526C>T                 | p.Gln217X                 | Hetero          |           |         |                           |                           |
| Nonsense  | 10      | c.8932C>T                 | p.Gln2978X                | CH              | Missense  | 8       | c.5356A>G                 | p.Asn1786Asp             |
| Nonsense  | 8       | c.4937C>A                 | p.Ser1646X                | Homo            | Nonsense  | 8       | c.4937C>A                 | p.Ser1646X                |
| Nonsense  | 8       | c.4937C>A                 | p.Ser1646X                | Homo            | Nonsense  | 8       | c.4937C>A                 | p.Ser1646X                |
| Exon deletion | 9   | c.7544-200_7677+1110del    | Homo Exon deletion        | 9               | c.7544-200_7677+1110del |
| Nonsense | 8 | c.4937C>A | p.Ser2646X | Hetero | Nonsense | 8 | c.6526C>T | p.Gln2176X |
|----------|----|------------|-------------|--------|----------|----|------------|-------------|
| Nonsense | 8 | c.6299C>A | p.Ser2100X | CH     | Nonsense | 16| c.10477C>T| p.Gln3493X |
| Nonsense | 8 | c.6299C>A | p.Ser2100X | CH     | Nonsense | 16| c.10477C>T| p.Gln3493X |
| Stop Codon | 16 | c.10769delC | p.Thr3590LysfsX6 | CH | Missense | 16 | c.11410C>T | p.Arg38404X |
| Exon deletion | 9 | c.7544-200_7677+1110del | Homo | Exon deletion | 9 | c.7544-200_7677+1110del |
| Nonsense | 8 | c.2041C>T | p.Arg681X | Homo | Nonsense | 8 | c.2041C>T | p.Arg681X |
| Nonsense | 8 | c.2041C>T | p.Arg681X | Homo | ?Nonsense | 8 | c.2041C>T | p.Arg681X |
| Exon deletion | 9 | c.7544-200_7677+1110del | Homo | Exon deletion | 9 | c.7544-200_7677+1110del |
| Nonsense | 16 | c.10483C>T | p.Gln3495X | CH | Frameshift | 16 | c.10775delC | p.Thr3592LysfsX6 |
| Nonsense | 16 | 11107C>T | p.Arg3703X | Hetero | |
| Frameshift | 10 | c.7911dupC | p.Asn2638Glnfs*24 | Homo | Frameshift | 10 | c.7911dupC | p.Asn2638Glnfs*24 |

CH indicates compound heterozygote; Hetero, Heterozygote; Homo, homozygote.

Table 3. Changes over time in markers
|                  | No. Patients | No. Scans | Correlation Analysis | GEE Analysis |
|------------------|-------------|-----------|----------------------|--------------|
|                  |             |           | Rho                  | p-Value      |
|                  |             |           | (95% CI)             | p-Value      |
| T1 SAX Basal (ms)| 30          | 104       | 0.415                | <0.001       |
|                  |             |           | 25.8 (20.0, 31.7)    | <0.001       |
| T1 SAX Mid (ms)  | 30          | 104       | 0.451                | <0.001       |
|                  |             |           | 21.8 (17.4, 26.1)    | <0.001       |
| T1 SAX Basal ECV | 30          | 94        | 0.181                | 0.014        |
|                  |             |           | 0.008, 0.020         | <0.001       |
| T1 SAX Mid ECV   | 30          | 94        | 0.192                | 0.011        |
|                  |             |           | 0.006, 0.016         | <0.001       |
| LVEDV Index (ml/m²) | 30          | 106       | -0.113               | 0.247        |
|                  |             |           | 0.04 (-0.60, 0.909)  | 0.68         |
| LVESV Index (ml/m² | 30          | 106       | -0.164               | 0.094        |
|                  |             |           | 0.92 (-0.06, 0.067)  | 1.90         |
| LVEF (%)         | 30          | 106       | 0.104                | 0.289        |
|                  |             |           | -0.27 (-1.23, 0.575) | 0.68         |
| LV Mass Index (g/m²) | 30          | 104       | 0.257                | 0.008        |
|                  |             |           | 2.8 (1.9, <0.001)    | 3.7          |
| LAV Biplane (ml/m²) | 29          | 60        | -0.221               | 0.090        |
|                  |             |           | -2.0 (-6.7, 4.09)    | 2.7          |
| GLS              | 30          | 95        | 0.032                | 0.756        |
|                  |             |           | -0.04 (-0.33, 0.809) | 0.26         |
| GRS              | 28          | 86        | 0.010                | 0.924        |
|                  |             |           | -0.36 (-1.18, 0.394) | 0.47         |
| E/A              | 29          | 72        | 0.133                | 0.267        |
|                  |             |           | 2.3 (-2.2, 6.9)      | 0.313        |
| E/e’ Average     | 30          | 68        | 0.161                | 0.189        |
|                  |             |           | 0.3 (-0.2, 0.299)    | 0.8          |
| HBA1c (mmol/mol) | 29          | 100       | -0.116               | 0.250        |
|                  |             |           | -0.9 (-2.5, 0.284)   | 0.7          |
| C-Peptide (pmol/L) | 30          | 94        | -0.028               | 0.788        |
|                  |             |           | -90.7 (364.6, 183.2) | 0.516        |
| Pro NT BNP (ng/L) | 30          | 105       | -0.101               | 0.306        |
|                  |             |           | 34.4 (33.9, 32.4)    | 102.8        |
Correlation analysis – results are from Spearman’s rho correlation coefficients between the marker and the timing of the scan, relative to the baseline scan.

GEE analysis - results are from generalised estimating equation models, as described in the methods. The “gradient” represents the average yearly change in the marker over the period.

Bold p-values are significant at p<0.05.

E/A, mitral early filling (E)/atrial filling (A); E/e’, mitral early filling (E)/early myocardial relaxation velocity (e’); ECV, extracellular volume; HbA1c, glycated haemoglobin A1c; LAV, left atrial volume; LV, left ventricular; LVEDV index, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESV index, left ventricular end-systolic volume indexed; NT-proBNP, serum Nterminal pro B natriuretic peptide; SAX, short axis.

| Table 4: Associations Between T1/ECV Over Time and Changes in Myocardial Structure and Function |
|-------------------------------------------------------------|
| Gradient in: | T1 SAX Mid Gradient | T1 SAX Mid ECV Gradient |
| N | Rho | p-Value | N | Rho | p-Value |
| LVEDV Index (ml/m²) | 26 | 0.019 | 0.927 | 24 | -0.052 | 0.809 |
| LVESV Index (ml/m²) | 26 | -0.444 | 0.023 | 24 | -0.188 | 0.379 |
| LVEF (%) | 24 | 0.352 | 0.091 | 22 | 0.088 | 0.699 |
| LV Mass Index (g/m²) | 26 | -0.056 | 0.784 | 24 | 0.203 | 0.340 |
| LAV Biplane (ml/m³) | 20 | 0.245 | 0.298 | 18 | -0.377 | 0.123 |
| GLS | 25 | 0.422 | 0.036 | 23 | 0.024 | 0.914 |
| GRS | 23 | 0.268 | 0.217 | 21 | 0.116 | 0.618 |
| E/A | 22 | -0.292 | 0.187 | 20 | -0.029 | 0.905 |
| E/e’ Average | 23 | -0.370 | 0.083 | 21 | -0.268 | 0.241 |
| HbA1c (mmol/mol) | 24 | -0.119 | 0.579 | 22 | 0.189 | 0.399 |
| C-Peptide (pmol/L) | 26 | -0.101 | 0.624 | 24 | -0.037 | 0.865 |
| Pro NT BNP (ng/L) | 26 | -0.072 | 0.726 | 24 | 0.023 | 0.913 |
For each of the markers considered, a linear regression model was produced for each patient, with the timing of the scan, relative to the first scan, set as a continuous covariate. Only those patients with at least two valid scans for the stated marker were included in the analysis. Spearman’s (rho) correlation coefficients were then produced between the resulting gradients. Bold p-values are significant at p<0.05.

E/A, mitral early filling (E)/atrial filling (A); E/e’, mitral early filling (E)/early myocardial relaxation velocity (e’); ECV, extracellular volume; HbA1c, glycated haemoglobin A1c; LAV, left atrial volume; LV, left ventricular; LVEDV index, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESV index, left ventricular end-systolic volume indexed; NT-proBNP, serum N-terminal pro B natriuretic peptide; SAX, short axis.

Table 5 – Association between baseline factors and changes in T1/ECV
|                       | T1 SAX Mid Gradient (ms per Year) | T1 SAX Mid ECV Gradient (per Year) |
|-----------------------|-----------------------------------|-----------------------------------|
| N                     | Mean±SD                           | p-Value                           |
| Age at First Scan*    | 26 -0.137* 0.505*                 | 24 0.169 0.430                    |
| Ethnicity             | 0.278                             | 0.360                             |
| White                 | 26.3 ±                             | 0.018 ±                           |
| British               | 25.2 17 0.025                      |
| Other                 | 12.7 ±                             | 0.007 ±                           |
|                      | 35.9 8 0.026                       |
| Gender                | 0.112                             | 0.864                             |
| Male                  | 15.5 ±                             | 0.014 ±                           |
| Female                | 34.5 ± 17 0.016 ±                 |
| ACE/ARB               | 0.969                             | 0.771                             |
| No                    | 6 21.7 ± 56.6                      |
| Yes                   | 20 22.2 ± 19 0.014 ±              |
| Statins/Fib rates     | 0.103                             | 0.121                             |
| No                    | 14 30.7 ± 14 0.021 ±              |
| Yes                   | 12 12.1 ± 10 0.005 ±              |
| Hypertension          | 0.930                             | 0.117                             |
| No                    | 11 21.5 ± 10 0.005 ±              |
| Yes                   | 15 22.5 ± 14 0.021 ±              |
| Hyperlipidaemia       | 0.040                             | 0.989                             |
| No                    | 11 35.5 ± 11 0.015 ±              |
| Yes                   | 15 12.3 ± 13 0.015 ±              |
| Infantile cardiomyopathy | 0.710                             | 0.904                             |
Gradients were calculated on a per-patient basis, as described in the methods, with the resulting values reported as mean±SD, and compared between groups using independent samples t-tests, unless stated otherwise. Only those patients with at least two valid scans for the stated marker were included in the analysis.

*Reported as a Spearman’s rho correlation coefficient and p-value. **Excludes N=3 patients with unknown CAD status

Bold p-values are significant at p<0.05

Table 6: Details of Seven Patients with Coronary Artery Disease or Coronary Artery Atheroma
| Patient ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-----------|---|---|---|---|---|---|---|
| Age       | 38| 42| 19| 48| 36| 43| 19|
| Gender    | M | M | M | M | M | F | M |
| Agatston  | PM| ICA| 1 | 48| 69| 156| 209 |
| Findings  | RCA Occlusion| PCI to LAD| Mild atheroma| Mild atheroma| Mild atheroma| Mild atheroma| LAD stenosis |
| LGE Pattern| Extensive diffuse| Focal epicardial| Mid wall LGE| Extensive diffuse| Mid wall LGE| Extensive diffuse| Focal epicardial |
| LGE territory| Basal inferolateral, basal, mid and apical lateral and inferior RV| Basal inferolateral, and mid inferolateral walls.| Basal inferolateral segment| Basal inferolateral and mid inferolateral transmural in the mid and apical anterior| Basal inferolateral| Basal and mid LV levels, mid antero-lateral, mid inferolateral, mid inferior segments| Mid inferior and mid infero-lateral LV segments. |
| HTN       | Y | Y | Y | Y | Y | Y | N |
| Hyperlipidemia| Y | Y | Y | Y | Y | Y | Y |
| Diabetes  | Y | Y | IR | Y | N | N | Y |
| CKD Stage | 2 | 5 | 0 | 2 | 1 | 1 | 2 |
| Infantile CM| N | Y | N | N | Y | Y | Y |

ICA, invasive coronary angiography; CKD, chronic kidney disease stage; CM, cardiomyopathy; HTN, hypertension; ID, identification number; LAD, left anterior descending; PM, Post mortem finding; N, no; Y, yes.

**Figures**
Figure 1

Serial Change Over Time. Figures represent the changes over time in the mid short axis of the left ventricle for A) T1 relaxation and B) extracellular volume fraction. In addition, plots C and D were produced to demonstrate the trajectories of T1 and ECV, respectively, for individual patients. The fit lines and gradients are from the general estimating equation models, as detailed in the methods, and use the intercept of the median patient. ECV indicates extracellular volume; SAX, short axis.