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Homozygous loss-of-function mutation in *ALMS1* causes the lethal disorder mitogenic cardiomyopathy in two siblings

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ABSTRACT

Background: Two siblings from consanguineous parents of Turkish descent presented with isolated dilated cardiomyopathy, leading to early death in infancy. The diagnosis of mitogenic cardiomyopathy was made histologically.
Methods and results: Linkage analysis combined with exome sequencing identified a homozygous deleterious mutation in the *ALMS1* gene as the cause of this phenotype.
Conclusions: Alström syndrome is characterized by a typically transient dilating cardiomyopathy in infancy, suggesting that mitogenic cardiomyopathy represents the extreme phenotype, resulting in demise before the other clinical symptoms become evident. This observation further illustrates the role of *ALMS1* and cell cycle regulation.

1. Introduction

Cardiomyopathies are a heterogenous group of primary myocardial disorders in which the heart muscle is structurally and functionally abnormal, in the absence of other causes including coronary artery disease, hypertension, valvular or congenital heart disease [Elliott et al., 2008].The annual incidence of pediatric cardiomyopathy is low, 1/100,000 children, with the highest incidence in the first year of life [Lipshultz et al., 2003; Nugent et al., 2003]. Four major types are distinguished, i.e., dilated, hypertrophic, restrictive, and arrhythmogenic right ventricular cardiomyopathy [Richardson et al., 1996]. Other unclassified types, which do not meet the criteria of one of the above, include endocardial fibroelastosis and ventricular non-compaction.

Mitogenic cardiomyopathy is an extremely rare type of dilated cardiomyopathy leading to death in early infancy. To date, only 8 cases have been reported in 5 families [Chang et al., 2010; Shenje et al., 2014; Zerbini et al., 1992]. Zerbini et al. described this condition in an 8-day-old infant, who died suddenly. Pathological examination revealed normal cardiac anatomy. The right ventricle was slightly dilated and endocardial fibroelastosis was present. Histology of the myocardium showed numerous mitoses and frequently enlarged myocardial nuclei with condensed chromatin forming a serrated thread running in the long axis, termed caterpillar nuclei. They observed an increased DNA ploidy of myocardial cells. The 1-month-old sibling of this patient also presented with heart failure and severe, dilated cardiomyopathy. An endomyocardial biopsy revealed endocardial fibroelastosis, but no increased mitoses. DNA ploidy analysis, on the other hand, showed an increased ploidy of the myocardial cells. This patient responded positively to intensive treatment; however, no long term follow-up data are available. In 2010 Chang et al. described 5 cases with an identical disorder, including 2 pairs of siblings. They all presented during early infancy with symptoms of cardiac failure and died soon thereafter. There were no associated extracardiac anomalies. Autopsy showed an enlarged, dilated heart, mostly ventricular, with endocardial fibroelastosis in all cases. Distinct findings were nuclear hypertrophy of the cardiomyocytes and a markedly increased mitotic activity with a proliferative index of 10–20% (normal < 1%), as well as caterpillar nuclei. In 1 of the 2 pairs of siblings there was parental consanguinity. This, and the observation of affected males and females strongly suggested autosomal recessive inheritance. More recently, in 2014, Shenje et al. described a proband and her sibling with neonatal heart failure requiring heart transplantation. Mitotic cardiomyocytes

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were observed. The parents were not consanguineous and cardiac evaluation of the parents were normal, leading to a hypothesis of e recessive disorder. Exome sequencing identified two heterozygous *ALMS1* mutations in the proband and her sibling, both of which result in frameshift and premature termination [Shenje et al., 2014]. We here report a novel family with an identical disorder. By a combination of linkage analysis and exome sequencing, we identify mutations in the *ALMS1* gene as the cause of this distinct type of cardiomyopathy.

2. Patient data

2.1. Patient 1

The index is the second child of healthy, consanguineous parents of Turkish descent. He was born at 41 weeks of gestation after an uneventful pregnancy. Weight was 3200 g (3rd–10th centile). He presented at age 20 days with excessive crying and feeding difficulties. On clinical examination, an inguinal hernia was noted. On reducing the hernia, cardio-circulatory arrest occurred. He was resuscitated and transferred to the university hospital. Despite continuous and prolonged resuscitation, the infant demised. Post-mortem echocardiography revealed a structurally normal heart.

The child was not dysmorphic. Weight was 4180 g (50th centile), length 56.5 cm (75th–90th centile) and head circumference 37.5 cm (50th centile). Pathological examination revealed signs of congestive cardiac failure (Fig. 1). The weight of the heart was 31.1 g (75th–5th centile) which is within normal range for age [Pryce et al., 2014]. There was cardiomegaly, caused by globular dilatation of the left ventricle [Guzeltas and Eroglu, 2012]. The endocardium was pale and thickened, indicative of endocardial fibroelastosis, which was confirmed histologically. Apart from this "dilated cardiomyopathy", the heart was structurally normal, including normal origin of the coronary arteries and normal aortic and mitral valves. There were no signs of noncompaction cardiomyopathy. Histology showed no signs of myocarditis, nor was there any evidence for a metabolic disorder. Myofibrillar disarray was absent. The most striking phenomenon was a marked mitotic activity in the cardiac myocytes (Fig. 2). The myocardium also showed myocyte nuclear hypertrophy with the frequent occurrence of binuclear and even trinuclear myocytes. Some myocytes contained caterpillar nuclei (Fig. 3), thus named due to condensed chromatin forming a serrated thread in the long axis. Immunohistochemical staining for Ki-67 (Mib1) showed a markedly increased proliferative activity of the myocardium. The proliferation index was 20% (normal value <1%).

These findings led to the diagnosis of mitogenic cardiomyopathy.

2.2. Patient 2

Because of the high recurrence risk, the following pregnancy was closely followed. Repeated prenatal cardiac ultrasound investigations remained normal. A female infant was born at 41 weeks gestation, weighing 2840 g (3rd–10th centile). Early neonatal echocardiography was normal. However, at day 19, she was admitted with overt heart failure. Echocardiography (Fig. 4) revealed a dilated cardiomyopathy with left ventricular inner dimension at end-diastolic (LVIDd) of 25 mm (normal 12.9–19.1 mm) [Guzeltas and Eroglu, 2012]. There was endocardial fibroelastosis and severe mitral and tricuspid regurgitation. Cardiac function was poor, with a fractional shortening (FS) of 12% (normal ≥30%) and retrograde pulmonary hypertension. Despite respiratory and circulatory support, she progressively deteriorated and demised at the age of 22 days. No autopsy was performed.

Family history was otherwise negative; cardiac investigations of both parents and the 5 year old sibling were normal (Fig. 4).

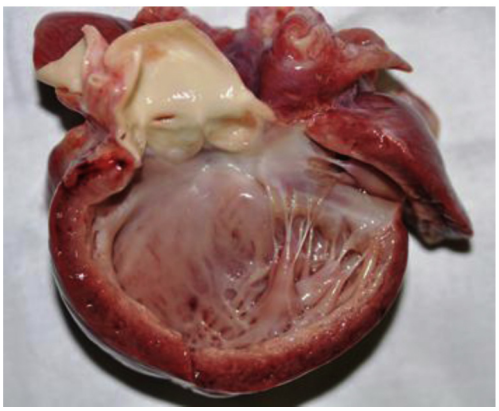

Fig. 1. This specimen (patient 1) of the left ventricle clearly shows left ventricular dilatation and endocardial fibroelastosis. The heart was structurally normal.

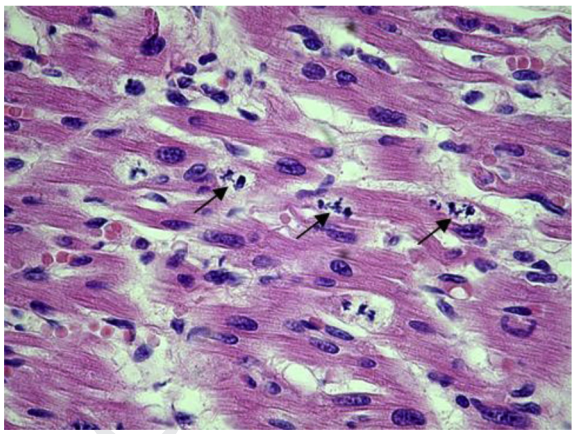

Fig. 2. This image shows markedly increased proliferative activity of the myocardium.

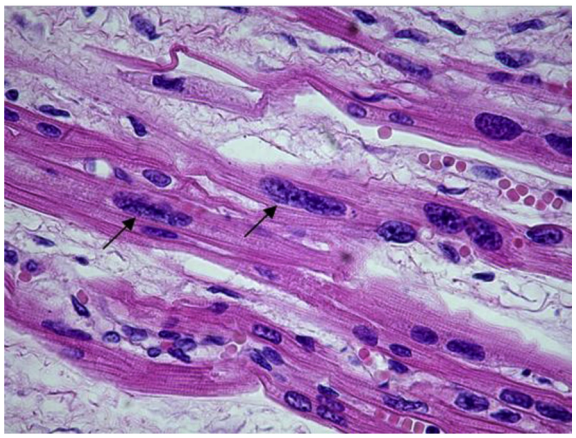

Fig. 3. Caterpillar nuclei with condensed chromatin.

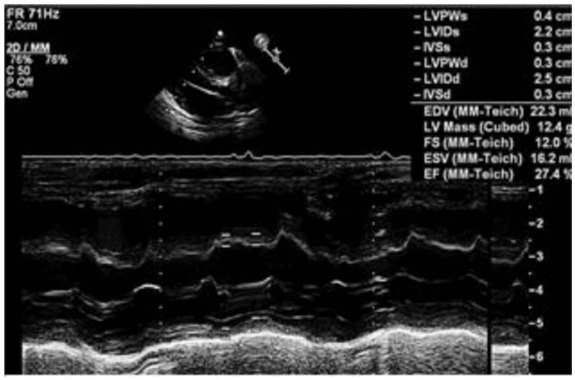

Fig. 4. Echocardiography using M-mode showing poor cardiac function (FS 12%, normal >30%) and dilated left ventricle. Left ventricle posterior wall at end- diastole (LVPWd), left ventricle inner dimension at end-diastole (LVIDd), Interventricular septum at end-diastole (IVSd), Left ventricular fractional shortening (FS).

Informed consent was given by the family for further genetic studies.

3. Methods

3.1. Linkage analysis

Genomewide parametric linkage analysis with Merlin software was performed [Abecasis et al., 2002]. A dense SNP marker set derived from the 250 k Affymetrics SNP typing platform was used in a recessive model. Genotyping was done on DNA extracted from peripheral white blood cells, obtained from the parents and both the unaffected and affected siblings.

3.2. Exome analysis

Q1 Library construction for all samples were prepared using *TruSeq DNA* Library Preparation Kit (Illumina, Inc., San Diego, CA, USA) in which platform-specific adapters and unique DNA indexes were ligated. For each sample, 1 μg genomic DNA was sheared by sonication to approximately 300 bp fragments, followed by end-repair, adenylation and adapter ligation steps. DNA sequencing libraries were subsequently enriched with the SeqCap EZ Human Exome Library v3.0 (Roche, NimbleGen), and 2 × 100-bp paired-end reads were generated on the Illumina HiSeq2000 platform with 3–4 exome-seq samples pooled per lane of a sequencing flow-cell. Sheared DNA, whole genome libraries and enriched exome-seq libraries were validated using DNA-1000 chips on the BioAnalyser (Agilent), and library concentrations were determined using the dsDNA Broad Range Assay using the Qubit (Invitrogen).

Data analysis was done using commercial and in-house developed software (Genomics Core/UZ Leuven). Exome sequences were obtained from both parents, patient 1 and the unaffected sister. Filtering was done with a High-quality depth of 5. From the variant files, we only retained variants in genes from the linkage regions. Additional mutations in all known hypertrophic and dilated cardiomyopathy genes were excluded. All homozygous calls were excluded in the parents and the unaffected sibling, reference calls were excluded in the affected sibling. According to Ensembl (www.ensemble.org) only exonic and splicing variants were included. Synonymous variants were excluded. Variants occurring with a frequency of <1% in the 1000 genomes project or with an unknown frequency were included. All remaining calls were checked for correct calling using Integrative Genomics Viewer (IGV, Broad Institute, Cambridge, MA, USA).

4. Results

4.1. Linkage analysis

In 8 regions the maximum LOD score of 1.9 was reached. All together these regions were spanning 76, 769 163 Mb and contained 487 HUGO genes.

4.2. Exome sequencing

After variant filtering as outlined in the methods section, we identified 6 candidate genes in the linkage region with homozygous mutations in the patient, inherited from both parents, and for which the unaffected sibling is heterozygous or reference (Supplementary Table 1S). This gene list was manually curated using functional data and genotype–phenotype correlations for the implicated genes. We thus identified a deleterious mutation in the *ALMS1* gene as the most likely cause. No additional mutations were found in known hypertrophic or dilated cardiomyopathy genes.

Results were confirmed by Sanger sequencing (Fig. 2S). The two affected siblings are homozygous for a frameshift deletion of one basepair in the *ALMS1* gene NM_015120.4:c.7760delG, p.Cys2587Phefs*5 (NC_000002.11:g.73716849delG or NG_011690.1:g.108964del). This is predicted to cause a premature stop at position 5 downstream. The unaffected sister and parents are heterozygotes.

5. Discussion

We report a novel family with an extremely rare and lethal disorder: mitogenic cardiomyopathy. It is characterized by infantile-onset dilated cardiomyopathy resulting in irreversible heart failure and death. Histologically, there is a dramatically increased mitotic activity in the cardiomyocytes. Given the likely autosomal recessive inheritance and parental consanguinity in this family, the combination of linkage analysis and exome sequencing allowed us to identify mutations in the *ALMS1* gene as the most likely cause of the condition in this family. The homozygous mutation in *ALMS1* was located in a 3, 435 478 MB region on chromosome 2 (Supplemental Fig. 1S).

The cardiac phenotype of dilated cardiomyopathy, although more severe, fits with Alström syndrome. In Alström syndrome, more than 60% of individuals develop congestive heart failure, most often dilated cardiomyopathy. An episode of heart failure due to dilated cardiomyopathy occurs in about 40% of cases during early infancy, between 2 and 16 weeks of age [Marshall et al., 2013]. In most cases the initial poor cardiac function improves and patients remain stable for many years. In about 15% a recurrence of restrictive heart failure occurs during adolescence or adulthood. In addition, 20% of patients present in adolescence or adulthood with progressive restrictive cardiomyopathy. Besides this interfamilial variability, pronounced intra-familial variability has been observed regarding the occurrence and severity of cardiomyopathy [Mahamid et al., 2013]. Thus, the severe dilated cardiomyopathy observed in the present family can be regarded as an extreme presentation of Alström syndrome. The clinical presentation in these infants is dominated by heart failure, at an age when the additional manifestations such as nystagmus were not yet evident. For this reason, the diagnosis of Alström was not suspected. Recently, *ALMS1* was identified in 6 affected infants with mitogenic cardiomyopathy [Shenje et al., 2014]. Our family further supports these findings and the importance of ALMS 1 in cell cycle regulation in perinatal cardiomyocytes.

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There is no immediate explanation for the more severe phenotype observed in this family. On the one hand, this may be due to ascertainment bias. On the other hand, the marked intra-familial variability, and interfamilial variability observed in individuals sharing the same *ALMS1* mutations suggests the presence of genetic and/or environmental modifiers [Marshall et al., 2007 Dec]. The mutation occurs in a region where many other mutations have been reported before, and, similarly to most of them result in a (predicted) truncated protein [Marshall et al., 2007 Nov]. Additional mutations in known hypertrophic or dilated cardiomyopathy genes were excluded.

The present observation indicates a link between *ALMS1* and the mechanisms underlying the neonatal structural and functional changes in the heart. In the different developmental stages from fetus to adult, the ventricular myocardium differs morphologically, quantitatively and qualitatively in function and structure. In the adult myocardium the myocytes are organized in parallel and there is little interstitial tissue between myocytes. Polyploidy is frequently noted and the nucleus is relatively small. In the fetal heart, myocytes are less well organized and the intercellular space is greater. Nuclei are large and polyploidy is unusual. In the immediate weeks following birth, a rapid decrease in myocyte mitosis is noted; essentially all growth beyond the early neonatal period is due to hypertrophy. The stimulus is the normal developmental increase in mural stress and work. The workload of the left ventricle increases postnatally due to increased left ventricular output. This causes a rapid increase in thickness and weight of the left ventricle due to an increase in myocytes [AM, 2009; Anderson et al., 2010].

The increased mitotic activity observed in cardiomyocytes in the present condition suggests a defect in cell cycle regulation. *ALMS1* localizes to centrosome and to the ciliary basal bodies. There is a well-established role of the primary cilium in regulating cell cycle [Basten and Giles, 2013]. Of interest, in a mouse model for Alström syndrome, carrying a homozygous truncating mutation in exon 10 (*alms1* $^{L2131x/L2131x}$), [Li et al., 2007] loss of cilia in the kidney was observed in older mice, associated with increased proliferation and cyst formation as well as apoptosis. In these mice, no cardiac phenotype was described. Also in the heart, cilia may play a role in cardiomyocyte development and proliferation. Abolishing the function of the primary cilium in the pluripotent mouse stem cells P19.CL6 prevents further differentiation of these cells into beating cardiomyocytes [Clement et al., 2009]. Recent studies further supports this hypothesis: two week old homozygous *ALMS1*-mutant mice show an increased cardiomyocyte proliferation. In *ALMS1* mRNA knockdown mice various proliferation markers are increased, as well as showing more cardiomyocytes in de G2/M phases. Furthermore, deficiency of the Alström protein in postnatal mice decreases postnatal cardiomyocyte cell cycle arrest. Phenotypically, the heart/body ratio was increased in mutant mice compared to wild-type [Shenje et al., 2014].

Reaching a genetic diagnosis in rare disorders remains a challenge. We illustrate that even in a single family with only two affected individuals, the identification of the underlying defect is feasible, using a combination of the sophisticated genetic tools. As in this family, we anticipate that the unbiased whole exome screens for mutation is likely to reveal further phenotypic heterogeneity in previously well delineated monogenic conditions.

Disclosures

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2014.06.004.

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