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A Mouse Model of Endocardial Fibroelastosis

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Abstract

Background—Endocardial Fibroelastosis (EFE) is a pathologic condition of abnormal deposition of collagen and elastin within the endocardium of the heart. It is seen in conjunction with a variety of diseases including hypoplastic left heart syndrome and viral endocarditis. While an experimental model using heterotopic heart transplant in rats has been described, we sought to fully describe a mouse model that can be used to further elucidate the potential mechanisms of and treatments for EFE.

Materials and Methods—The hearts of 2-day-old C57BL/6 mice were transplanted into the abdomen of 7-week-old C57BL/6 mice. At 2 weeks, the hearts were harvested and histologic analysis performed using hematoxylin and eosin, Masson's Trichrome, Russell-Movat's Pentachrome, Picrosirius Red, Hart's, Verhoeff-Van Gieson, and Weigert's Resorcin-Fuschin stains. Additionally, one heart was analysed using transmission electron microscopy (TEM).

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Disclosures

The authors have no other conflicts of interest to disclose.

Results—Specimens demonstrated abnormal accumulation of both collagen and elastin within the endocardium with occasional expansion in to the myocardium. Heterogeneity in extracellular matrix deposition was noted in the histologic specimens. In addition, TEM demonstrated the presence of excess collagen within the endocardium.

Conclusions—The heterotopic transplantation of an immature heart into a mouse results in changes consistent with EFE. This model is appropriate to investigate the etiology and treatment of endocardial fibroelastosis.

Keywords

Endocardial fibroelastosis; EFE; Unloaded heterotopic heart transplant; Mouse model; Congenital heart disease

1. Introduction¹

Endocardial fibroelastosis (EFE) is a pathologic condition characterized by expansion of the endocardium by collagen and elastin (Figure 1). On gross examination, these changes appear as a pearly or opaque white inner lining of the heart, predominately in the ventricles [1, 2]. The thickening caused by the abnormal deposition of collagen and elastin within the smooth muscle layer of the endocardium is thought to limit ventricular diastolic compliance and might also restrict ventricular growth [3, 4].

EFE is reported in conjunction with a broad spectrum of diseases (Table 1), including hypoplastic left heart syndrome (HLHS) and viral endocarditis [1], which have been comprehensively reviewed in the literature. Little is known about the pathogenesis of this condition, in part due to the wide variety of circumstances in which it occurs. Some authors have suggested that an inflammatory response within the heart leads to a proliferation and migration of mesenchymal cells such as fibroblasts, myofibroblasts, or smooth muscle cells that deposit extracellular matrix constituents such as collagen and elastin within the endocardium [1, 4, 5].

While EFE has been described in a variety of animals such as horses, dogs, cats, ruminants, and chickens [6–10], the spontaneity with which these changes occur makes these species suboptimal for rigorous investigation. A rat model of EFE involving the heterotopic transplant of a 2-day old donor heart into the abdomen of a syngeneic recipient rat has previously been described. Using the concept that low-flow induces endocardial remodeling, the authors demonstrated that unloaded heterotopic transplant recapitulates some of the changes seen in EFE [11]. The aim of the current study was to apply these concepts to a mouse model, which is better suited to elucidate the cellular and molecular mechanisms behind this complex pathology.

¹EFE – Endocardial fibroelastosis; HLHS – Hypoplastic left heart syndrome; PBS – phosphate buffered saline; H&E – hematoxylin and eosin; VVG – Verhoeff-Van Gieson; WRF – Weigert’s Resorcin-Fuschin; TEM – transmission electron microscopy

2. Materials and Methods

2.1 Animal Care / Ethics Statement

The Institutional Animal Care and Use Committee of the Research Institute at Nationwide Children's Hospital (Columbus, OH) approved and monitored the protocol for the present study. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (2011), from the Public Health Service, National Institutes of Health (Bethesda, MD).

2.2 Donor Heart Harvest

Two day-old C57BL/6 pups (Jackson Laboratories, Bar Harbor, ME) (n=18) were euthanized by intraperitoneal injection of ketamine (300mg/kg; Hospira Inc., Lake Forest, IL) and xylazine (30 mg/kg; Akon Inc., Decatur, IL), followed by induced pneumothorax. The vena cava (left and right cranial, caudal), pulmonary artery, and aorta were identified, isolated, ligated, and transected. The heart was then placed in phosphate buffered saline (PBS) (Gibco R, Life Technologies) on ice until the time of implantation (<10 minutes).

2.3 Unloaded Heterotopic Heart Transplantation

Two-day old donor hearts were implanted into the peritoneal cavity of seven week-old C57BL/6 mice (n=18) in an unloaded configuration as previously described [12]. The ascending aorta was anastomosed to the infrarenal aorta, and the pulmonary artery was anastomosed to the infrarenal vena cava (Figure 2). In rats, this model has previously been shown to receive a preload of approximately 5% of the circulating blood volume to the transplanted heart [11]. Perfusion of the heart occurs via the coronary arteries to the coronary sinus and into the right atrium.

2.4 In-Vivo Imaging of Heterotopic Heart Implants

Two weeks or 3 months post-implantation, mice underwent echocardiographic assessment with pulsed-wave Doppler ultrasound (30 MHz, VisualSonics, Inc.). Anesthesia was induced with 1.5% isoflurane (Baxter, Deerfield, IL) vaporized with oxygen at a flow rate of 1 L/min. To eliminate poor visualization due to intestinal gas or rotation of the implanted heart, the midline incision was re-opened to eviscerate the intestines and expose the transplanted heart graft. The peritoneal cavity was filled with 0.3mL of warm normal saline and covered with a sterile impermeable, transparent membrane. Long and short axis images were acquired in B-mode, after which mice were euthanized.

2.5 Histology

Mice were euthanized with an overdose cocktail of ketamine (300mg/kg) and xylazine (30 mg/kg), followed by induction of pneumothorax. Systemic vasculature was perfused via apical puncture of the native heart with PBS (Gibco, Life Technologies) followed by 10% neutral buffered formalin (Fisher). The heterotopic hearts were harvested and fixed in 10% neutral buffered formalin at 4° C overnight, and submitted for routine processing and paraffin embedding. Serial sections (4 µm thick) were stained with hematoxylin and eosin (H&E), Masson's Trichrome, Russell-Movat's Pentachrome (American MasterTech, Lodi,

CA), Picrosirius Red (15.31 ms exposure under polarized light), Hart's, Verhoeff-Van Gieson (VVG), and Weigert's Resorcin-Fuschin (WRF) (Long Method, Electron Microscopy Sciences) stains. All stains were evaluated for specificity and sensitivity with internal positive controls when available or positive and negative control slides from isogenic control mice stained in parallel.

2.6 Ultrastructural characterization

Sacrifice was performed as above excluding whole body perfusion with formalin. One heart was fixed in 10% neutral buffered formalin and 3% glutaraldehyde in sodium cacodylate buffer (pH 7.4) (Electron Microscopy Sciences). Samples underwent conventional processing for electron microscopy by the Comparative Pathology and Mouse Phenotyping Shared Resource (The Ohio State University, Columbus, OH). Trimmed sections (50 nm) were placed on copper grids and stained with filtered lead citrate/sodium citrate solution (Electron Microscopy Sciences) for 5 minutes. Grids were imaged on a JEOL JEM-1400 TEM (JEOL USA, Inc.) and imaged with an Olympus SIS Veleta 2K camera (Olympus Soft Imaging Solutions GmbH).

3. Results

3.1 Surgical Outcomes and Echocardiographic Findings

Of the 18 mice that underwent heterotopic transplant, overall survival was 50% ($n = 7$ (39%) survived until the 2 week time point and $n = 2$ were survived until 3 months (11%)). Of these 9 hearts, six underwent routine processing for histology at 2 weeks, 1 underwent processing for transmission electron microscopy (TEM) at 2 weeks, and 2 were processed for routine histology at 3 months. The majority of deaths occurred early in the series and within 24 hours of implantation, with a survival rate of 80% among the last 5 mice. The echocardiogram prior to heterotopic heart explantation demonstrated viability in terms of blood flow and pulsatility, in addition, left ventricle hypertrophy was appreciated (Figure 3, Supplemental video 1).

3.2 Histological Analysis

The morphology of heterotopic hearts was compared to that of native hearts of both 2-day old mice ($n=3$) and 2-week old mice ($n=3$) using H&E staining (Figure 3). While there was a small amount of somatic growth in the heterotopic hearts by histomorphometry, their size was more comparable to the native 2-day hearts at the time of explant. In all heterotopic hearts, there was thickening of the left ventricular myocardium and endocardial expansion due to an increase in extracellular matrix constituents.

Collagen Deposition—Collagen deposition was evaluated using Masson's Trichrome, Russell-Movat's Pentachrome, and Picrosirius Red stains (Figure 4). Masson's Trichrome and Russell-Movat's Pentachrome stains demonstrated pathologic fibroplasia of the left ventricular endocardium within the heterotopic hearts. Further, Picrosirius Red staining imaged by polarized light microscopy exhibited a population of collagen fibers with heterogeneous thickness and alignment (green-yellow to yellow-orange birefringence). In

contrast to the 2-week post implantation hearts that show a predominance of thin fibers (green-yellow), the 3-month samples reveal increased thick collagen fibers (yellow-orange).

Elastin Deposition—Elastin deposition within the left ventricular endocardium was demonstrated by VVG, Hart's, and WRF stains (Figure 5). Both VVG and Hart's stains exhibit elastin fibers underlying the endothelium in all samples. This finding was confirmed by visualization of fibers in this same region on both the WRF and Russell-Movat's Pentachrome stains. While organized linear, laminated elastin fibrils were appreciated in some regions of the 2-week post-implantation heterotopic hearts, disorganized and immature elastin fibers were more prevalent within the left ventricular endocardium. However, 3 month post-implantation heterotopic hearts demonstrated increased thickness, organization, and alignment of the elastin fibers.

Heterogeneity of Changes—While each heart demonstrates both elements of abnormal collagen and elastin deposition within the endocardium, there is heterogeneity in the distribution and pattern of pathologic remodeling (Figure 6). In all cases, the process involved the left ventricle, and in one case there was involvement of the intraventricular septum with disease involving both ventricles.

3.3 Ultrastructural Characterization

Fibroplasia of the subendocardial extracellular matrix was demonstrated within the left ventricle of the heart processed for TEM (Figure 7). While the majority of collagen is observed in cross-section, a few segments of collagen are in longitudinal orientation, and demonstrate axial periodicity. The collagen is interpreted as mature, organized fibrils with uniform shape and size surrounded by abundant clear space, interpreted as edema; elastin was not identified in the evaluated sections. Cells within this matrix demonstrate abundant rough endoplasmic reticulum, suggesting ongoing protein synthesis (Figure 7b).

4. Discussion

The clinical management of endocardial fibroelastosis is impacted by the relative paucity of data regarding the underlying biomechanical, cellular, and molecular mechanisms of disease progression. Currently, medical treatment is directed at symptomatic management of heart failure, although there are reports of prenatal treatment of EFE with steroid therapy [13, 14]. Surgical resection of the endocardium has also been attempted in a small subset of patients, but has a high morbidity even in a select population and may result in conduction abnormalities [15–17]. While a previous report has described a rat model, our study is the first to fully characterize changes consistent with EFE in a mouse, offering the use of a vast array of molecular reagents (i.e. transgenics) that are only readily available in mice and allowing for further rigorous investigation into the cellular and molecular mechanisms underlying EFE.

The previous heterotopic transplantation of neonatal rat hearts into adult rats demonstrated some of the changes associated with EFE by using an unloaded heterotopic model in which perfusion of the heart occurs via the coronary vessels with low intraventricular flow, mimicking many congenital heart diseases. A loaded model, in comparison, allowed

antegrade flow through the left side of the heart via a surgically-created connection between the pulmonary artery and left atrium [11]. Using these models, this study demonstrated the importance of low-flow to the development of EFE.

While the previous rat model of EFE clearly demonstrated an excess deposition of collagen, characterization of abnormal elastin deposition was limited. Evidence of both excess collagen and elastin deposition within the endocardium is necessary to fully replicate remodeling consistent with EFE. In each of the six hearts presented here, both characteristics were demonstrated through histologic staining, exhibiting changes similar to those observed in human specimens. However, there was a noted heterogeneity in the distribution of disease with expansive collagen deposition. Many of the specimens demonstrate either immature or disorganized elastin deposition. While this disorganization is inconsistent with more mature and severe forms of EFE, some human clinical specimens of early disease display similar findings [18]. TEM confirmed the excess deposition of collagen within the endocardium at a subcellular level, but the elastin observed with histologic staining was not as clearly evident. This might be a consequence of the limited amount of tissue examined in the TEM studies or site selection artifact.

A potential weakness of this model is the differences in endocardial microanatomy between the mouse and human. Murine endocardium is limited in mass due to a scant connective tissue layer. In humans, the endocardial space is relatively more complex, consisting of the extracellular matrix, smooth muscle cells, capillaries, and unmyelinated nerves [20, 21]. Despite these differences, the changes observed in the mouse model are consistent with those appreciated in human specimens. This may suggest a shared histogenesis for EFE between these models.

The technical complexity and associated mortality are also limitations of the model. However, with experience, acceptable mortality (20%) was achieved within the final 5 transplantations. The ability to modulate flow by altering the vascular anastomosis represents a strength of the model as these changes may impact the heterogeneity of EFE changes within human patients. While this model may not represent such varied causes of EFE like viral endocarditis or dilated cardiomyopathy, a commonality between these etiologies is low flow dynamics. Our model may best represent conditions of altered flow dynamics as in hypoplastic left heart syndrome. In addition, the transplantation itself may contribute to the inflammatory process and changes seen in this model. Based on previous work in a rat model, it is unlikely that the operation is the source of the changes, as demonstrated by the lack of EFE in a loaded model [11].

Several studies have suggested that mechanobiological pathways, which would be impacted by low flow, play an important role in the expression of various genes associated with endothelial to mesenchymal transition [19–22]. Furthermore, a recent article using heterotopic heart transplant in a mouse model suggested that endothelial to mesenchymal transition may be responsible for the changes seen in EFE [5]. While genetic predisposition has been used to explain the heterogeneity in EFE [18], it may be that differences in flow contribute to phenotypic variability. With the characterization of a mouse model of EFE and the assistance of transgenic mouse models, the mechanisms and treatment of these changes

can be interrogated, leading to targeted therapies to halt or reverse the changes associated with EFE.

5. Conclusion

An unloaded heterotopic heart transplant in a mouse model leads to changes consistent with endocardial fibroelastosis observed clinically in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Lurie PR. Changing concepts of endocardial fibroelastosis. *Cardiology in the young*. 2010; 20:115–123. [PubMed: 20346203]
2. Perloff, JK.; Marelli, AJ. *Clinical Recognition of Congenital Heart Disease*. Sixth Edition ed.. Philadelphia, PA: Elsevier Saunders; 2012. Endocardial Fibroelastosis; p. 141-146.
3. McElhinney DB, Vogel M, Benson CB, Marshall AC, Wilkins-Haug LE, Silva V, et al. Assessment of left ventricular endocardial fibroelastosis in fetuses with aortic stenosis and evolving hypoplastic left heart syndrome. *The American journal of cardiology*. 2010; 106:1792–1797. [PubMed: 21126622]
4. Neustein HB, Lurie PR, Fugita M. Endocardial fibroelastosis found on transvascular endomyocardial biopsy in children. *Archives of pathology & laboratory medicine*. 1979; 103:214–219. [PubMed: 582252]
5. Xu X, Friehs I, Zhong Hu T, Melnychenko I, Tampe B, Alnour F, et al. Endocardial fibroelastosis is caused by aberrant endothelial to mesenchymal transition. *Circulation research*. 2015; 116:857–866. [PubMed: 25587097]
6. Cushing TL. Endocardial fibroelastosis in a quarterhorse mare. *Journal of comparative pathology*. 2013; 149:318–321. [PubMed: 23651694]
7. Bentley DM. Congenital endocardial fibroelastosis in a dog. *The Canadian veterinary journal La revue veterinaire canadienne*. 1999; 40:805–807. [PubMed: 10563242]
8. Pass DA. A cardiomyopathy ("sudden death syndrome") of adult hens. *Avian pathology : journal of the WVPA*. 1983; 12:363–369.
9. Scarratt WK, Sponenberg DP, Welker FH, Keith JC Jr, Gardner D. Endocardial fibroelastosis and tricuspid valve insufficiency in a calf. *Journal of the American Veterinary Medical Association*. 1987; 190:1435–1436. [PubMed: 3610756]
10. Zook BC, Paasch LH. Endocardial fibroelastosis in Burmese cats. *The American journal of pathology*. 1982; 106:435–438. [PubMed: 7065121]
11. Friehs I, Illigens B, Melnychenko I, Zhong-Hu T, Zeisberg E, Del Nido PJ. An animal model of endocardial fibroelastosis. *The Journal of surgical research*. 2013; 182:94–100. [PubMed: 22938709]

12. Asfour B, Hare JM, Kohl T, Baba HA, Kass DA, Chen K, et al. A simple new model of physiologically working heterotopic rat heart transplantation provides hemodynamic performance equivalent to that of an orthotopic heart. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation*. 1999; 18:927–936.
13. Steger CM, Antretter H, Moser PL. Endocardial fibroelastosis of the heart. *Lancet*. 2012; 379:932. [PubMed: 22217671]
14. Raboisson MJ, Fouron JC, Sonesson SE, Nyman M, Proulx F, Gamache S. Fetal Doppler echocardiographic diagnosis and successful steroid therapy of Luciani-Wenckebach phenomenon and endocardial fibroelastosis related to maternal anti-Ro and anti-La antibodies. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2005; 18:375–380. [PubMed: 15846168]
15. Emani SM, McElhinney DB, Tworetzky W, Myers PO, Schroeder B, Zurakowski D, et al. Staged left ventricular recruitment after single-ventricle palliation in patients with borderline left heart hypoplasia. *Journal of the American College of Cardiology*. 2012; 60:1966–1974. [PubMed: 23062531]
16. Czosek RJ, Atallah J, Emani S, Hasan B, del Nido P, Berul CI. Electrical dyssynchrony and endocardial fibroelastosis resection in the rehabilitation of hypoplastic left cardiac syndrome. *Cardiology in the young*. 2010; 20:516–521. [PubMed: 20529397]
17. van Son JA. Ross-Konno operation with resection of endocardial fibroelastosis for critical aortic stenosis with borderline-sized left ventricle in neonates. *The Annals of thoracic surgery*. 1997; 63:1834. [PubMed: 9230038]
18. Hutchins GM, Vie SA. The progression of interstitial myocarditis to idiopathic endocardial fibroelastosis. *The American journal of pathology*. 1972; 66:483–496. [PubMed: 5060581]
19. Egorova AD, Khedoe PP, Goumans MJ, Yoder BK, Nauli SM, ten Dijke P, et al. Lack of primary cilia primes shear-induced endothelial-to-mesenchymal transition. *Circulation research*. 2011; 108:1093–1101. [PubMed: 21393577]
20. Ten Dijke P, Egorova AD, Goumans MJ, Poelmann RE, Hierck BP. TGF-beta signaling in endothelial-to-mesenchymal transition: the role of shear stress and primary cilia. *Science signaling*. 2012; 5(pt2)
21. Frueh J, Maimari N, Homma T, Bovens SM, Pedrigi RM, Towhidi L, et al. Systems biology of the functional and dysfunctional endothelium. *Cardiovascular research*. 2013; 99:334–341. [PubMed: 23650287]
22. Groenendijk BC, Hierck BP, Gittenberger-De Groot AC, Poelmann RE. Development-related changes in the expression of shear stress responsive genes KLF-2, ET-1, and NOS-3 in the developing cardiovascular system of chicken embryos. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2004; 230:57–68. [PubMed: 15108309]

Figure 1. Endocardial Fibroelastosis in an infant heart

Comparison of normal human endocardium (**a, b**) and endocardium with EFE (**c, d**).

Photomicrographs demonstrate expansion of the endocardium with collagen and elastin (**c, d**). Masson's Trichrome (**a, c**); collagen (blue), myofibers (red); Verhoeff-Van Gieson Stain (**b, d**): elastin (fibrillar basophilic stain). V: Ventricle; M: Myocardium.

Figure 2. Low-flow heterotopic transplantation of murine heart into the abdomen

(a): Diagram of low-flow heterotopic transplantation of murine heart into the abdomen of adult mouse. Anastomoses: Donor pulmonary artery to the caudal vena cava, donor ascending aorta to infrarenal aorta. Ligated vessels: caudal vena cava, cranial vena cava. **(b):** Intraoperative image of anastomoses demonstrating perfusion of the heart. LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle.

Figure 3. Comparison of 2-day and 2-week-old native hearts to heterotopic transplant hearts Low magnification photomicrographs and echocardiograms of 2-day (**a, d**) and 2-week (**b, e**) native hearts compared to heterotopic hearts (**c, f**) at 2 weeks post-implantation. The heterotopic hearts were noted to have only a small amount of somatic growth over the 2 week implantation period (scale bar **a** and **c**: 500 µm; scale bar for **b**: 1000 µm). Echocardiograms (**d, e, f**) also demonstrated relative left ventricular hypertrophy and thickening of the endocardium (red arrow). LV: left ventricle.

Figure 4. Histologic demonstration of collagen deposition

Photomicrographs of native 2-day, native 2-week, and heterotopic transplanted hearts (2 weeks and 3 months) using Masson's trichrome (**a, d, g, j**): collagen (blue), myofibers (red); Russell-Movat's pentachrome (**b, e, h, k**): nuclei/elastin (black), collagen (yellow), ground substance (blue), myofibers/fibrin (red); Picrosirius red (**c, f, i, l**): collagen (yellow-orange to green birefringence).

Figure 5. Histologic demonstration of elastin deposition

Photomicrographs of native 2-day, native 2-week, and heterotopic transplanted hearts using Verhoeff-Van Gieson (**a, d, g, j**): nuclei/elastin (blue to black), collagen (red); Hart's (**b, e, h, k**): elastin (blue-black), collagen (red), cytoplasm (yellow), nuclei (blue); Weigert's Resorcin-Fuchsin (**c, f, i, l**): elastin (blue-black), collagen (pink-red), nuclei (pale blue-black).

Figure 6. Heterogeneity of endocardial changes

Photomicrographs of 6 heterotopically transplanted hearts stained with Russell-Movat's pentachrome demonstrating the variation in distribution of the endocardial changes (a-f). RV: right ventricle; LV: left ventricle.

Figure 7. Transmission electron microscopy (TEM) images of heterotopic heart

Images taken at the level of the endothelial-subendothelial layers of the left ventricular wall.

(a): Demonstrates cells, collagen (*), and edema are constituents of the expanded subendocardial layer. Dashed line denotes endothelial basement membrane. (inset, a): Periodicity of collagen fibrils. (b): Cell within ECM demonstrating abundant RER within the cytoplasm and closely apposed collagen fibrils. ECM: extracellular matrix; E: endothelial cell; V: ventricular lumen; RER: rough endoplasmic reticulum.

Table 1

Diseases and other stressors associated with endocardial fibroelastosis.

<i>Cardiomyopathies</i>	<i>Myocarditis</i>
<i>Dilated</i>	<i>Viral</i>
Post-myocarditis	Mumps
Genetic	Coxsackie
Known genes	Adenovirus
Muscle LIM protein	<i>Bacterial</i>
C-linked fetal cardiomyopathy	Lactobacillus
Beta cardiac myosin heavy chain	<i>Lysosomal storage diseases</i>
Familial but idiopathic	<i>Glycogen</i>
Autosomal recessive	Type II Pompe
Autosomal dominant	<i>Mucopolysaccharides</i>
X-linked recessive	Hurler's
<i>Hypertrophic</i>	Maroteaux-Lamy
Noonan syndrome (left atrium)	<i>Sphingolipids</i>
<i>Restrictive</i>	Niemann-Pick's
Post-myocarditis	<i>Gangliosides</i>
Idiopathic	Infantile Sandhoff's
<i>Non-compaction</i>	<i>Other metabolic</i>
Barth syndrome	Systemic carnitine deficiency
<i>Congenital malformation</i>	<i>Physical injury</i>
Aortic stenosis	Post-electric shock
Coarctation of the aorta	<i>Vascular</i>
Anomalous coronary arteries	Myocardial infarction
Hypoplastic left heart syndrome	Twin-twin transfusion
Intracranial arteriovenous fistula	Lymphatic obstruction
<i>Immunologic disease</i>	
Transplacental maternal antibodies	
Rhesus incompatibility	

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