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Case Report

Spontaneous hypertrophic cardiomyopathy in a cynomolgus macaque (*Macaca fascicularis*)

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Abstract: The term *cardiomyopathy* is used to describe heart disease resulting from an abnormality in the myocardium. It is rare in cynomolgus macaques (*Macaca fascicularis*). Here, we report a case of hypertrophic cardiomyopathy in an 11-year-old male cynomolgus macaque. Macroscopically, the interventricular septum (IVS) and the left ventricular (LV) and right ventricular (RV) walls of the heart were thickened. Histologically, cardiomyocytes showed hypertrophy and disarray with interstitial fibrosis, and some myocytes showed karyomegaly and vacuoles. On the basis of these morphological characteristics, the present case was diagnosed as hypertrophic cardiomyopathy. Immunohistochemically, the cardiomyocytes in the affected regions were positive for the autophagic markers LC3 and p62/SQSTM1 (p62). The accumulation of autophagosomes in hypertrophied cardiomyocytes was demonstrated. The mechanism of accumulation of autophagosomes seems to be a secondary effect due to stress. To our knowledge, this is the first report of spontaneous hypertrophic cardiomyopathy in a cynomolgus macaque. (DOI: 10.1293/tox.2017-0027; J Toxicol Pathol 2018; 31: 49–54)

Key words: cynomolgus macaque, hypertrophic cardiomyopathy, disarray, vacuolation, autophagy

The term *cardiomyopathy* is used to describe heart disease resulting from an abnormality in the myocardium[1]. In the field of veterinary medicine, cardiomyopathies are divided into five morphologic types: hypertrophic, dilated (congestive), restrictive, arrhythmogenic right ventricular (RV), and unclassified[2]. The gross morphological features of each type are as follows: thickening of the left ventricular (LV) wall and interventricular septum (IVS) with narrowing of the lumen in the case of hypertrophic cardiomyopathy, rounded heart because of biventricular dilation in the case of dilated (congestive) cardiomyopathy, endocardial thickening because of fibrosis in the case of restrictive cardiomyopathy, enlargement of the RV chamber in the case of arrhythmogenic RV cardiomyopathy, and left or bilateral atrial dilation with normal LV wall thickness in the case of unclassified cardiomyopathy[2]. Even though incidental small foci of myocardial degeneration frequently occur in cynomolgus macaques (*Macaca fascicularis*), a diagnosis of cardiomyopathy is rare[3–5]. Here, we report a case of spontaneous hypertrophic cardiomyopathy in a male cynomolgus macaque.

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The animal had been purchased from Guangxi Grandforest Scientific Primate Company Ltd. (Dayiling Pingnan County, Guangxi, China) and was individually housed in our animal facility in a stainless steel cage (625 mm × 660 mm × 780 mm) in an environmentally controlled room with a temperature range of 20–26°C, a relative humidity of 35–75%, and a light:dark cycle of 12:12 h. PS or PS-A commercial feed (Oriental Yeast Co., Ltd., Tokyo, Japan) was provided daily, and tap water was available *ad libitum*. The study was conducted in compliance with the Internal Regulations on Animal Experiments at Nippon Shinyaku Co., Ltd., which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as revised on 1 June 2006). The animal had been repeatedly administered small amounts of test substances in pharmacokinetic tests between 4 and 9 years of age. It showed no abnormal clinical findings and no significant changes in routine blood tests. At 11 years of age, the animal was euthanized by exsanguination via the abdominal aorta under deep pentobarbital anesthesia.

For histological examination, tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin (HE). In addition to HE, heart sections were stained with Masson's trichrome (MT) and phosphotungstic acid hematoxylin (PTAH). For immunohistochemistry, heart sections were stained with antibodies by a labeled-polymer reagent method using an EnVision+ System (Dako Inc., Tokyo, Japan). The primary antibodies used are shown in Table 1.

Table 1. Primary Antibodies Used in this Study

Antibody	Clone	Dilution	Antigen retrieval[a]	Source[b]
Anti-CD-68	KP1	1:50	Heat	Dako
Anti-LC-3	Polyclonal	1:500	Heat	MBL
Anti-p62/SQSTM1	Polyclonal	1:500	Heat	MBL

[a]Heat: potassium citrate buffer (pH 6.0), 98°C, 40 min. [b]Dako: Glostrup, Denmark. MBL: Nagoya, Japan.

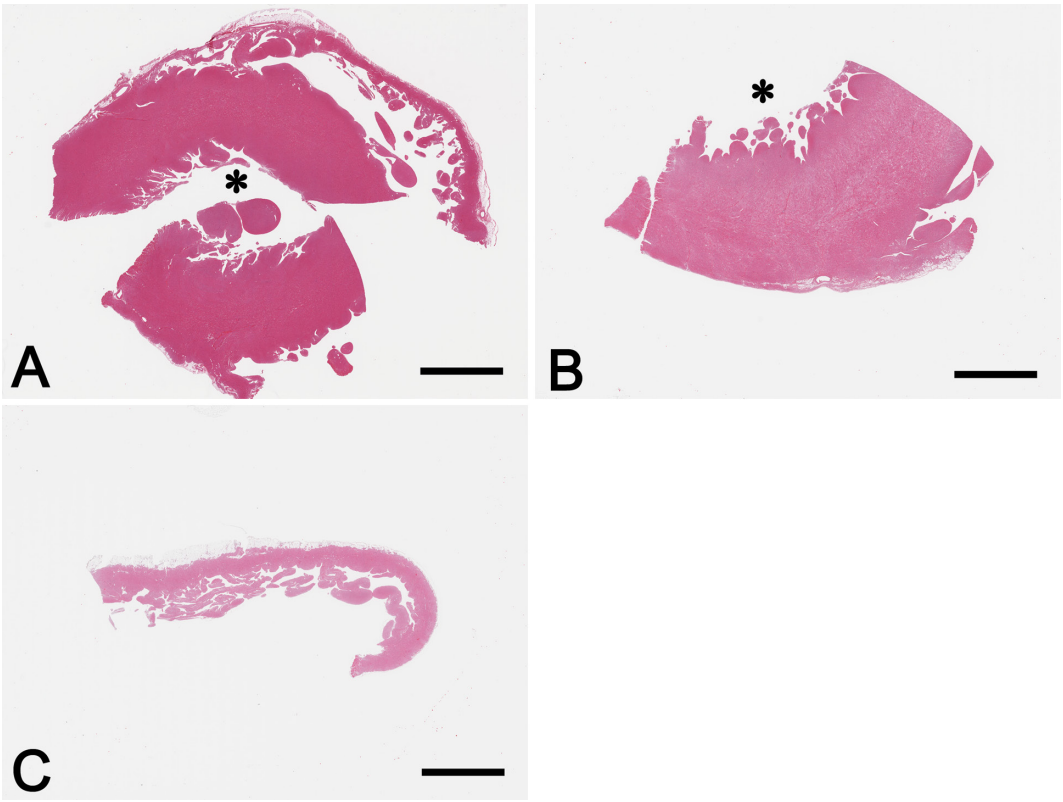

Fig. 1. Transverse sections of the hearts of an age-matched normal animal (A) and the present case (B and C). In comparisons with the age-matched normal animals (A), the IVS and LV wall (B) were moderately thicker, and the RV wall (C) was slightly thicker. The asterisk indicates the LV cavity. HE stain. Bar = 5 mm.

For electron microscopic examination, small pieces of the LV of the heart fixed in 10% neutral buffered formalin were refixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon resin. Ultrathin sections were stained with uranyl acetate and lead acetate and examined under a transmission electron microscope. However, useful information could not be obtained by this method because of the long period of storage in 10% neutral buffered formalin.

In comparisons with age-matched normal animals that had been purchased at the same time from the same facility and treated similarly, including pharmacokinetic tests and necropsy, macroscopically, the IVS and LV wall were moderately thicker, and the RV wall was slightly thicker (Fig. 1). Histologically, the lesions exhibited hypertrophy, disarray and vacuolation of cardiomyocytes with atypical karyomegaly, moderate interstitial fibrosis, and minimal inflammatory cells (Fig. 2 and 3). These changes were seen in the median muscular layer of the LV wall and/or IVS. Cardiomyocytes and nuclei were markedly hypertrophied in the LV wall and the IVS (Fig. 2). Cardiomyocyte disarray was marked in the IVS (Fig. 3B), though none was observed in the LV wall. MT staining indicated that fibrotic tissue with a high collagen content had spread in the LV wall and IVS (Fig. 3). Cardiomyocytes containing large vacuoles were observed in fibrotic areas prominently in the LV wall and slightly in the IVS (Fig. 2B, 3B and 3D). Vacuoles were also scattered among the collagen fibers, which seemed to be the remnants of disrupted cardiomyocytes (Fig. 3D). The RV wall showed mild hypertrophy of cardiomyocytes with mild interstitial fibrosis. With PTAH staining, there was a scattered decrease or loss of the blue staining of the cardiomyocytes, indicating degeneration of myocardial striation. Inflammatory cells were positive for the macrophage marker

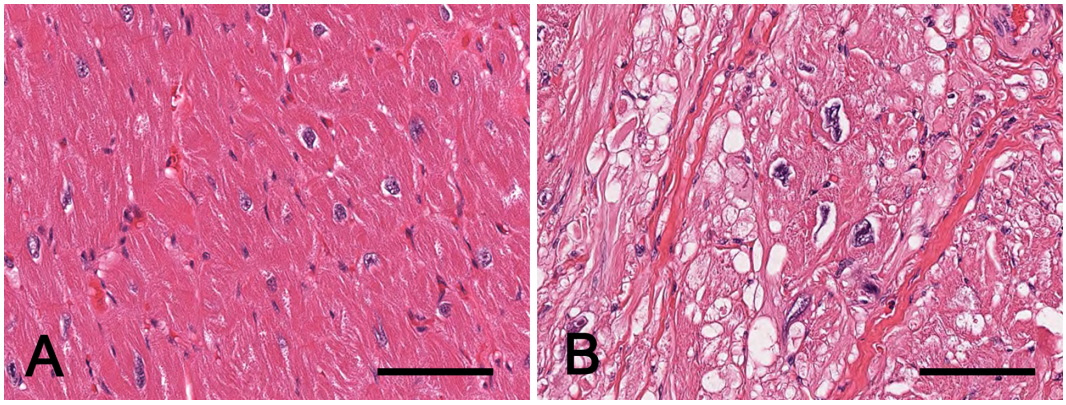

Fig. 2. Photomicrographs of sections of the LV wall of an age-matched normal animal (A) and the present case (B). (A) The LV wall shows no abnormality. (B) Hypertrophy, vacuoles, and atypical karyomegaly of cardiomyocytes with interstitial fibrosis. HE stain. Bars = 100 µm.

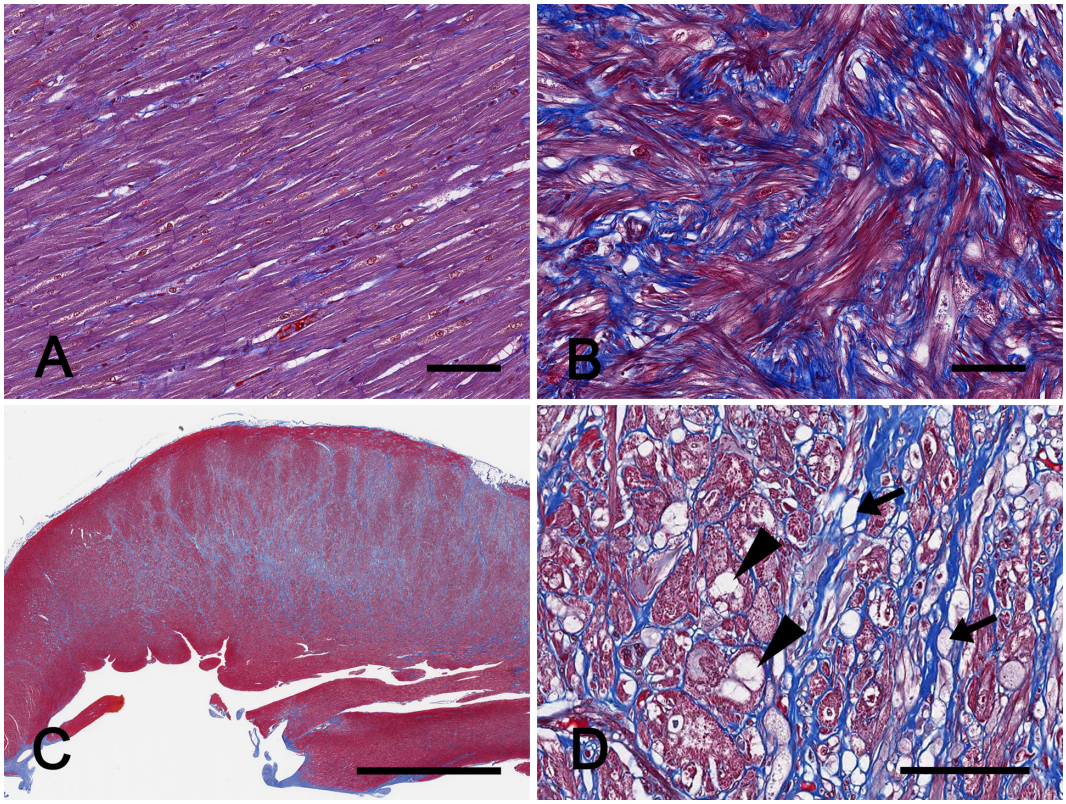

Fig. 3. Photomicrographs of sections of the heart of an age-matched normal animal (A) and the present case (B–D). (A) The IVS shows no abnormality. (B) Disarray of cardiomyocytes in the IVS. (C) Blue-stained fibrous tissue was spread throughout the median muscular layer of the LV wall. (D) Prominent interstitial fibrosis in the LV wall. Large vacuoles were observed in the cytoplasm of cardiomyocytes (arrowheads) and collagen fibers (arrows). MT stain. Bars = 100 µm (A, B, D) or 0.5 mm (C).

CD68. The cytoplasm of cardiomyocytes was granular positive for LC3 and p62 in the LV wall (Fig. 4C and 4D) and IVS (Fig. 4E and 4F), while the large vacuoles were negative for both markers. Cells that were positive for LC3 and p62 were frequently observed in the area where vacuoles of cardiomyocytes and fibrosis were abundant (Fig. 4C–4F). In the IVS, cardiomyocytes that were positive for LC3 and p62 were less scattered than in the LV wall, since vacuoles in fibrotic areas were less frequently observed in the IVS (Fig. 4E and 4F). Hearts of age-matched normal animals were negative for LC3 and p62 (Fig. 4A and 4B). Changes associated with heart lesions were not observed in other organs.

Hypertrophic cardiomyopathy is caused by sarcomeric defects in cardiomyocytes[2]. The LV wall and IVS thickens, and mild thickening of the RV wall is occasionally present[2].

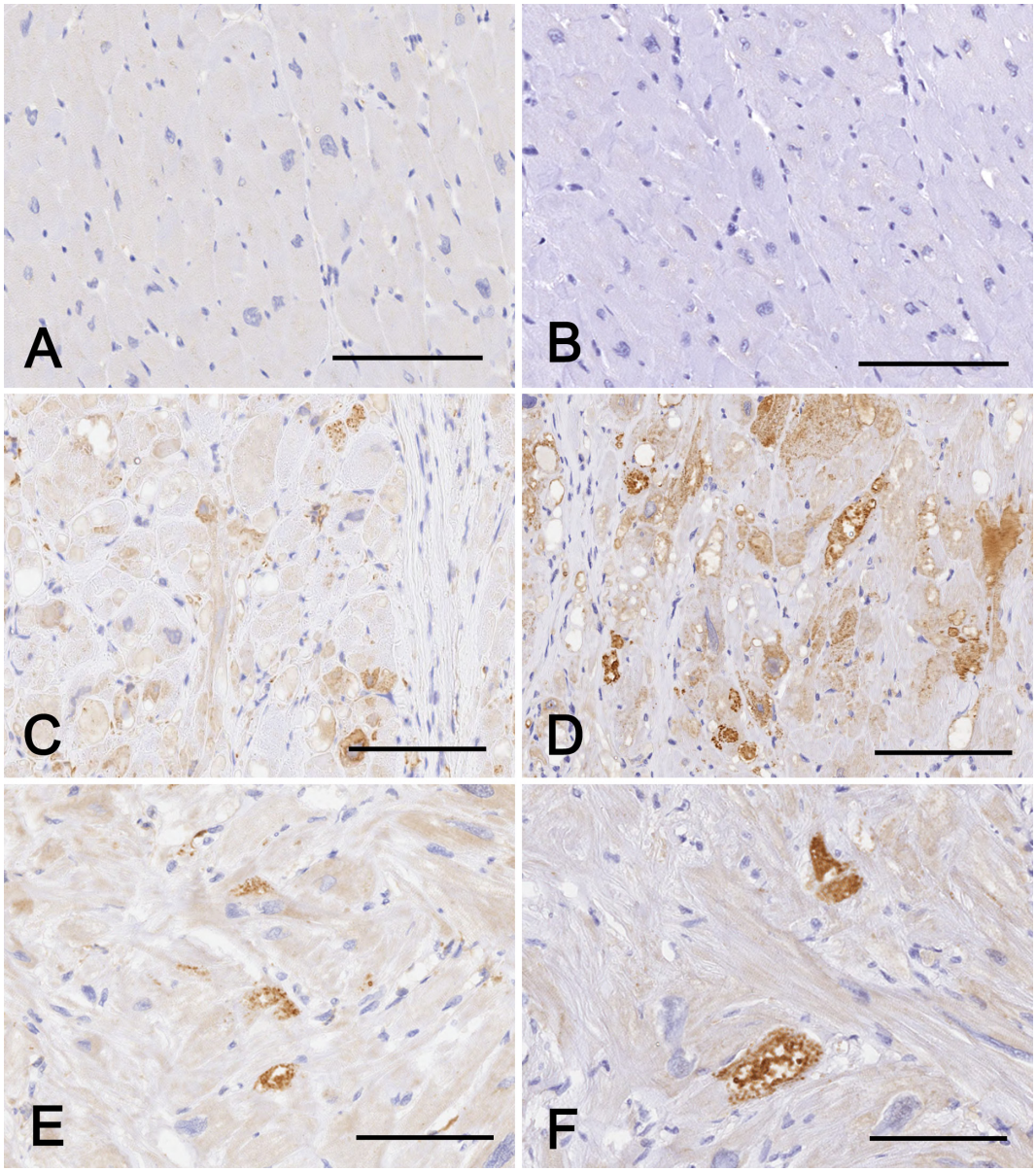

Fig. 4. Immunohistochemical staining of the heart of an age-matched normal animal (A and B) and the present case (C–F). The cytoplasm of cardiomyocytes in the LV wall showed granular positive for LC3 (C) and p62 (D), while the large vacuoles were negative for both. The cytoplasm of cardiomyocytes in the IVS showed granular positive for LC3 (E) and p62 (F). The LV wall of an age-matched normal animal was negative for LC3 (A) and p62 (B). Bars = 100 µm.

Altered sarcomeric function ultimately results in cardiomyocyte hypertrophy, collagen synthesis, and cardiomyocyte disarray[2]. On the basis of the morphological characteristics, that is, macroscopic thickening of the IVS and the LV and RV walls, histological hypertrophy and disarray of cardiomyocytes, and fibrosis in the heart, the present case was diagnosed as hypertrophic cardiomyopathy. Various substances had been administered to the animal for pharmacokinetic tests, but drugs inducing cardiac hyperfunction or causing toxicity changes to the heart had not been used. Furthermore, the hearts of the two age-matched normal animals used for similar tests showed no significant changes. Therefore, the present case was considered to be a spontaneous lesion.

The characteristic histopathological findings of human hypertrophic cardiomyopathy are the same as those of animals and include hypertrophy and disarray of cardiomyocytes and fibrosis in the heart[6]. In human hypertrophic cardiomyopathy, cardiomyocyte disarray is most prominent in the IVS of cases with asymmetric septal hypertrophy[7]. Our monkey case, which showed these findings, is thought to have shown characteristics similar to those of human hypertrophic cardiomyopathy.

The differential diagnosis of hypertrophic cardiomyopathy in humans includes inherited lysosomal storage diseases that could show LV hypertrophy and vacuoles of

cardiomyocytes such as Danon disease[8, 9] and Fabry disease[9]. Danon disease, caused by mutation of *LAMP2*, which encodes lysosome-associated membrane protein, shows cardiac hypertrophy and skeletal myopathy related to the accumulation of autophagic vacuoles[6, 8]. Fabry disease results from a deficiency of the enzyme α-galactosidase and progressive lysosomal deposition in cells throughout the body, and its signs and symptoms include severe pain in the extremities, the appearance of vascular cutaneous lesions, corneal and lenticular opacities, and the deterioration of renal function[6, 9]. These diseases were excluded in the present case because there were no abnormalities in clinical signs, skeletal muscles, the skin, the eyes, or the kidneys. In hypertrophic cardiomyopathy in humans, more than 400 mutations in 11 sarcomeric genes encoding contractile proteins of the cardiac sarcomere have been described, and many cardiomyopathies are known to be familial[10]. However, the family history of the present case is unknown.

In cynomolgus macaques, several cases have been diagnosed as cardiomyopathy, but none of them have been diagnosed as hypertrophic cardiomyopathy. Most cardiomyopathies previously reported in cynomolgus macaques have been characterized by the degeneration or necrosis of cardiomyocytes with karyomegaly and inflammation or fibrosis[3–5]. Cardiomyocyte disarray and vacuolation of the perimysial connective tissue have been observed in four cases of spontaneous cardiomyopathy in cynomolgus macaques[3]. However, there were no gross heart changes in those cases, and there was no mention of histological hypertrophy of cardiomyocytes[3].

Chamanza *et al.*[4] reported that idiopathic myocardial degeneration and cardiomyopathy are characterized by hypertrophy and vacuolation of cardiomyocytes. This is the only report that we know of that describes vacuolation of cardiomyocytes in cardiomyopathy of cynomolgus macaques, but a detailed description of the vacuoles was not given. In the present case, vacuoles of cardiomyocytes were more frequently observed in the area where cells that were positive for LC3 and p62 were abundant, but the vacuoles themselves were negative for both LC3 and p62, and their origin could not be identified under an electron microscope.

Autophagy is a lysosomal degradation pathway that is essential for survival, differentiation, development, and homeostasis[11]. Autophagy principally plays an adaptive role to protect organisms against diverse pathologies, including infections, cancer, neurodegeneration, aging, and heart disease[11]. An increase in lipidated LC3-labeled autophagosomes and/or p62 aggregates is a robust marker of autophagic flux inhibition at any point beyond autophagosome formation[12]. Autophagy occurs under normal conditions and can be further stimulated by different stressors[11, 13]. When cardiac stresses are sustained for long periods of time, cardiomyocytes remodel their cellular architecture (e.g., undergo elongation and hypertrophy) to adapt to the stress[11]. The needs of the stressed heart for more energy substrates and for cellular remodeling as described above may be met in part through the autophagy pathway[11]. In humans, autophagic vacuolar cardiomyopathy induced by CQ or HCQ is characterized by prominent cytoplasmic vacuolation that is ultrastructurally identified as secondary lysosomes and shows immunoreactivity for LC3 and/or p62[14]. Both CQ and HCQ accumulate within lysosomes, resulting in lysosomal alkalization[15] and the inhibition of lysosomal hydrolytic enzymes[16], and the concomitant inhibition of autophagy can lead to the accumulation of cellular debris and misfolded protein aggregates[11]. In the present case, it was obvious from the immunoreactivities of LC3 and p62 in cytoplasm without large vacuoles that autophagosomes had accumulated in cardiomyocytes, suggesting the existence of autophagosome formation abnormalities. However, we could not discover immunohistochemically or ultrastructurally whether or not the vacuoles in cardiomyocytes were autophagic vacuoles. Additionally, the distributions of the cardiac lesion and LC3/p62 positivity did not coincide. Therefore, the accumulation of autophagosomes in the present case may just be a sign indicating damage to cardiomyocytes due to the stress of hypertrophic cardiomyopathy. Autophagosome formation may have increased secondarily.

Takotsubo cardiomyopathy is known in humans as left ventricular apical ballooning syndrome and emotional or physical stress-induced cardiomyopathy[17]. In acute biopsy, electron microscopy revealed that numerous vacuoles of different sizes were present in cardiomyocytes[18]. The diagnostic criteria of Takotsubo cardiomyopathy includes the absence of other pathological conditions to explain the pattern of temporary LV dysfunction observed (e.g., hypertrophic cardiomyopathy)[17]. This disease was excluded in the present case because there was obvious cardiac hypertrophy.

To our knowledge, this is the first report of spontaneous hypertrophic cardiomyopathy in a cynomolgus macaque. This case of hypertrophic cardiomyopathy in a monkey was pathologically similar to that in humans, but details of the causes, including its heritability, are unknown. Additionally, this case showed accumulation of autophagosomes in cardiomyocytes, which seems to be a secondary effect due to stress, but again the details are unknown. Further studies of multiple cases are needed to clarify the pathophysiology of cardiomyopathy in cynomolgus macaques, including the mechanism of accumulation of autophagosomes and the identity of vacuoles in the cardiomyocytes.

Disclosure of Potential Conflicts of Interest: The authors declare that there are no conflicts of interest.

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