

# Ultrastructural Alterations in Acquired Aortic and Mitral Valve Disease as Revealed by Scanning and Transmission Electron Microscopical Investigations

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**Scanning and transmission electron microscopical investigations were carried out on explanted human aortic and mitral valves to study the prevalence of hyperplastic and degenerative lesions in acquired valvular dysfunction. Biopsies were taken from 67 aortic and 23 mitral valves. All of the valves examined showed degenerative lesions including a loose binding of the endothelial cells, a partial denudation of the endothelial cover and areas of fibrous hyperplasia surrounded by calcium deposits. Additionally, the formation of various excrescences was detected by means of scanning electron microscopy. Of all excrescences identified, 90% were localized at the free margin of the leaflet, 3% in the subnodular region and 7% in the nodule of Arantius. The ratio of filiform to lamellar forms of hyperplastic lesions was approximately 80% in most of the samples examined. The results presented demonstrate the complex ultrastructural features of surgically explanted human valves showing both degenerative and hyperplastic lesions in the same valve. (Ann Thorac Cardiovasc Surg 2002; 8: 24–30)**

**Key words:** scanning and transmission electron microscopy, human aortic and mitral valves, excrescences

## Introduction

Degenerative lesions of heart valves are often associated with valvular stenosis or insufficiency resulting in hemodynamic deterioration. Atherosclerotic degeneration has been identified as a main cause of valvular dysfunction finally leading to valve replacement surgery.<sup>1-4)</sup> Our present knowledge of endothelial changes in degenerative valve disease has, for the main part, resulted from investigations on post mortem or surgically explanted tissues.<sup>5-8)</sup> Generally, mechanical influences have been addressed as a major cause of endothelial lesions in degenerative valve disease. In contrast to the degenerative lesions, our knowledge of hyperplastic lesions to date has

been chiefly restricted to Lambl's excrescences which have usually been described in aortic or mitral valves.<sup>7,9-12)</sup> These hyperplastic alterations in the endothelium have commonly been regarded as primary benign cardiac tumors, however, the etiology of these lesions is still not known.<sup>9-12)</sup> Lambl's excrescences seem to occur in up to 90% of healthy subjects.<sup>10)</sup> The aim of this study was to evaluate the ultrastructural changes in human valves by means of a systematic scanning and transmission electron microscopical investigation on surgically explanted aortic and mitral valves. In particular, we were interested to determine the prevalence of hyperplastic lesions in degenerative valve disease.

## Materials and Methods

### Collection of specimens

For this study, samples from human aortic and mitral valves were taken from patients undergoing a valve replacement operation at the Clinic for Thoracic, Heart and Vascular Surgery. From each valve a leaflet was removed.

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**Table 1. Baseline characteristics of the study population**

	Aortic valve disease (n=67)	Mitral valve disease (n=23)
Valve pathology		
Stenosis	36 (53.7%)	8 (34.8%)
Insufficiency/combined	31 (46.3%)	15 (65.2%)
Male	n = 28	n = 18
Female	n = 39	n = 5
Age (mean $\pm$ SD)		
Gender		
Male	73 $\pm$ 3	71 $\pm$ 4.5
Female	68 $\pm$ 5	72 $\pm$ 8
NYHA		
I/II	13 (19.4%)	4 (17.4%)
III/IV	54 (80.6%)	19 (82.6%)
Concomitant diseases		
CHD	41 (61.2%)	14 (60.9%)
Syncope	11 (16.4%)	0
Embolism	2 (3.0%)	1 (4.3%)
Cardiac failure	16 (23.9%)	9 (39.1%)
Hypertension	50 (74.6%)	11 (47.8%)
Endocarditis	4 (6.0%)	0
Rheumatic fever	2 (3.05%)	0
Surgical procedure		
Isolated VR	26 (38.8%)	9 (39.1%)
VR + CABG	41 (61.2%)	14 (60.9%)
Combined VR	9	9

Continuous variables are presented as mean  $\pm$  standard deviation. Categorical variables are presented as an absolute percentage.

CABG: coronary artery bypass graft, CHD: coronary heart disease, NYHA: New York Heart Association, SD: standard deviation, VR: valve replacement.

Biopsies were taken from 67 aortic and 23 mitral valves, as shown in Table 1. The aortic valves were from 28 male (average age; 73 $\pm$ 3 years) and 39 female patients (68 $\pm$ 5 years). The study population included patients suffering from aortic stenosis (n=36, 53.7%) and aortic insufficiency (n=31, 46.3%). The mitral valves were removed from 5 female (72 $\pm$ 3 years) and 18 male patients (71 $\pm$ 4.5 years). There were 15 cases of mitral insufficiency (65.3%) and 8 cases of mitral stenosis (34.7%). Table 1 summarizes the clinical data including the comorbid diagnoses and the operations that had been performed.

### Scanning electron microscopy

Scanning electron microscopy was performed according to standard procedures. Briefly, specimens from the leaflets of human aortic and mitral valves were fixed for six hours in a solution containing 2.5% glutaraldehyde and 200 mM cacodylate. Samples were dehydrated in an as-

ending series of alcohol and dried using a critical point drier. All samples were then sputtered with gold-palladium. For electron microscopical observation, a digital scanning microscope (DSM 960, Zeiss) was used.

### Transmission electron microscopy

Transmission electron microscopy was performed to reveal ultrastructural details of the different valves. Specimens from valve leaflets were fixed for two hours in a solution containing 2% glutaraldehyde and 100 mM cacodylate at pH 7.3. The samples were then washed in phosphate-buffered saline and postfixed for two hours in cacodylate buffer containing 2% osmium tetroxide. The specimens were dehydrated in a graded series of alcohol and embedded in Araldite. Ultrathin sections were stained with 2% methanolic uranyl acetate. Samples were then examined in a transmission electron microscope equipped with photo documentation.



**Fig. 1.** Macroscopic aspect of a surgically explanted valve showing severe aortic stenosis and a rough surface of the leaflets.

## Results

### Scanning electron microscopical results

The surgically explanted human aortic and mitral valves were frequently associated with ultrastructural alterations of the endothelial cover. In the extracellular matrix characteristic ultrastructural features were detected. In all valves examined, areas of loose binding of the cells to each other and to the underlying basement membrane were observed. The endothelial cells were often separated from each other into small isolated islands (Fig. 2a). In these areas the endothelial cells were sometimes clumped together to form columnar lines which were regarded as transitional structures on the way to the formation of Lambl's excrescences. Numerous excrescences were identified at the edges of the leaflets and in areas of attachment of the aortic and mitral rings. Filiform and lamellar excrescences could be distinguished from one another, although some excrescences showed overlapping ultrastructural features (Fig. 2b, c). At higher magnification, sometimes a partial denudation of the endothelial layer was seen. The endothelial cells still attached to the excrescences appeared swollen and had only loose contacts

to each other (Fig. 2d). At the base of the excrescences, there was a transition from sparsely distributed superficial endothelial cells into underlying collagen fibers. The excrescences occurred in the nodule of Arantius, subnodular and at the free margin of the leaflets. The ratio of filiform to lamellar forms was approximately 80% in most of the samples tested. Excrescences located in the free-margin region of the leaflet were observed in 90%, in subnodular localization in 3% and in the nodule of Arantius in 7% of all cases. However, in the samples taken from patients suffering from endocarditis (n=4) no excrescences were found. Table 2 summarizes the distribution of the different types of excrescences among the valves examined.

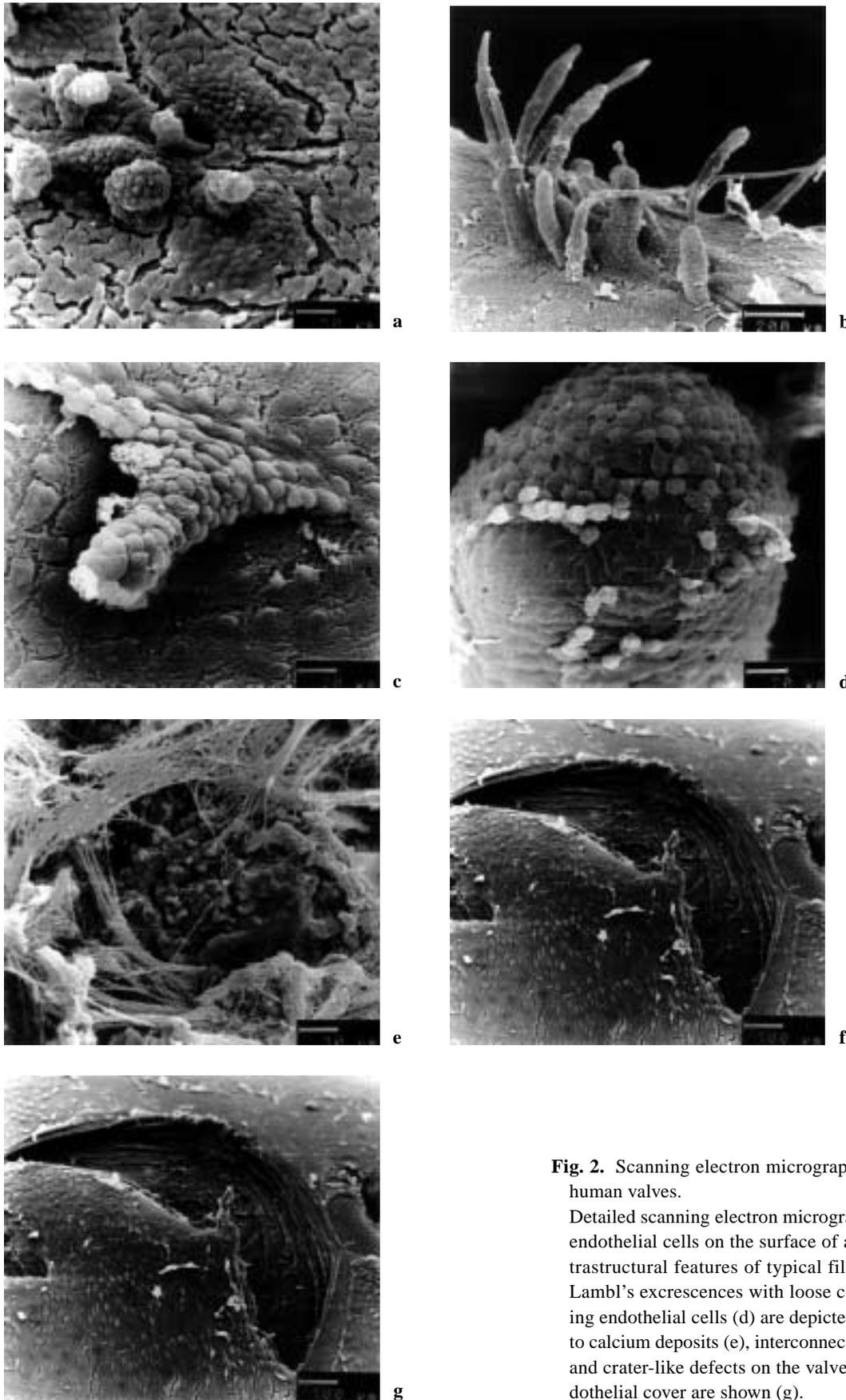
Furthermore, locally well defined elevations emerging from the intact endothelium were detected. At higher magnification, the endothelium showed clear tearing. When the calcium deposits, of which the elevations sometimes consisted, were removed, collagen fibers organized in multilayers were identified (Fig. 2e). The calcium deposits enclosed within hollow spaces were apparently isolated by interconnecting bundles of collagen fibers (Fig. 2f). Infrequently, crater-like defects were recognized on the surfaces of some valves which at higher magnification can be seen to have an endothelial cover. In these areas, the endothelial cells displayed similar ultrastructural alterations as described for the other parts of the valves (Fig. 2g). Notably, no relationship was found between the observed changes and the type of valvular disease.

### Transmission electron microscopical results

In order to study the ultrastructure of the explanted valves, transmission electron microscopy was performed. The results showed numerous collagen fibers adjacent to Lambl's excrescences. Only rarely cells were identified in the interior of the valves. Surprisingly, the endothelial cover was often missing. Long stretches of the basement membrane were exposed to the blood stream (Fig. 3a). An investigation of Lambl's excrescences showed that they consisted of several layers of collagen fibers. Here

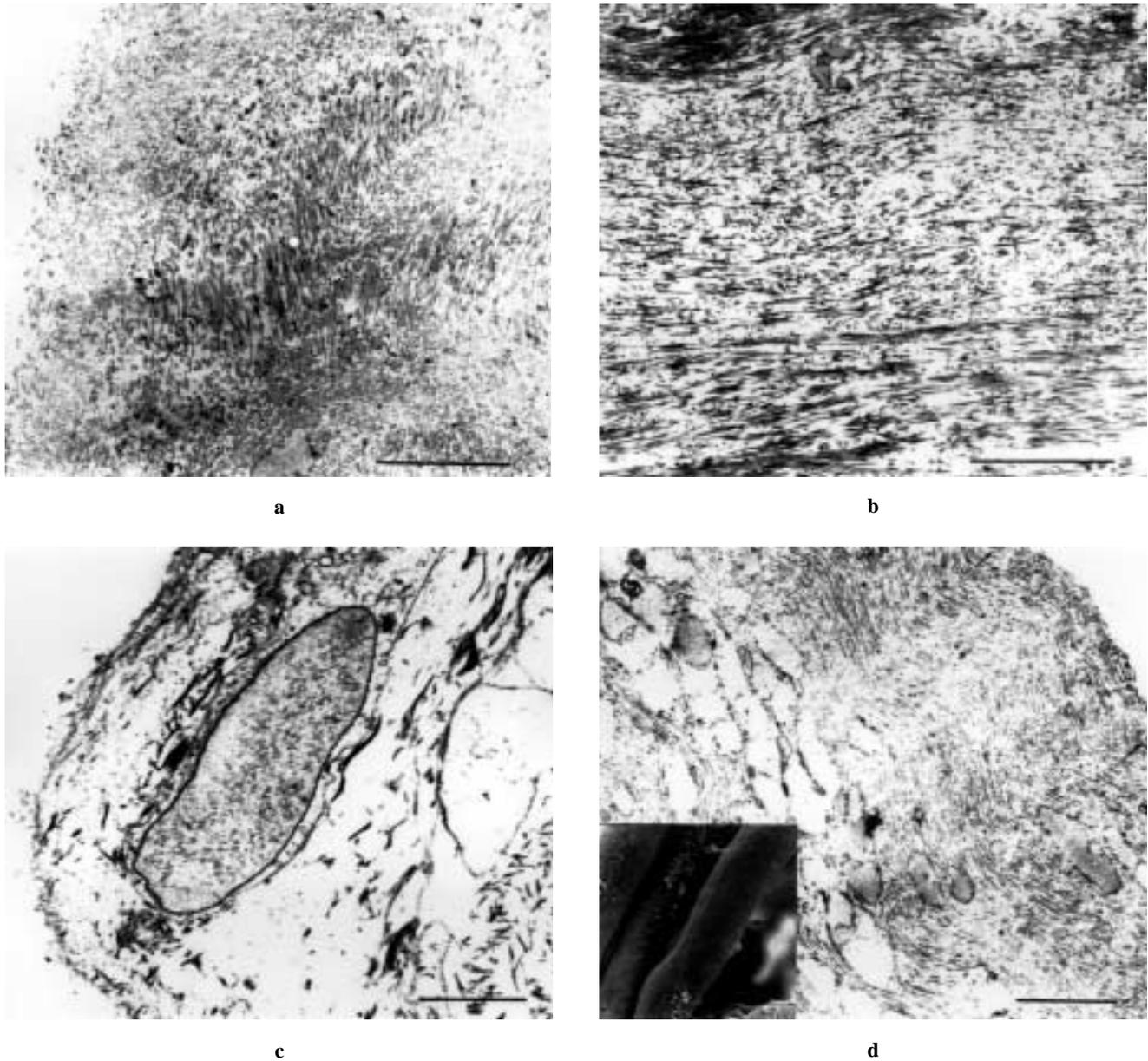
**Table 2. Distribution of Lambl's excrescences in non-cardiac deaths (according to Hurle et al. 1986) and in our study population of patients with valve disease**

	Nodule of Arantius	Subnodular region	Free margin of the leaflet
Non-cardiac deaths	94%	36%	22%
Study patients	7%	3%	80%



**Fig. 2.** Scanning electron micrographs of surgically explanted human valves.

Detailed scanning electron micrographs demonstrating altered endothelial cells on the surface of an explanted valve (a). Ultrastructural features of typical filiform (b) and lamellar (c) Lambl's excrescences with loose contacts between neighboring endothelial cells (d) are depicted. Collagen fibers adjacent to calcium deposits (e), interconnecting bundles of collagen (f) and crater-like defects on the valve surface disrupting the endothelial cover are shown (g).



**Fig. 3.** Transmission electron micrographs of valvular lesions.

Transmission electron micrograph showing detachment of endothelial cells and exposure of the underlying basement membrane to the blood stream (a). Interwoven layers of collagen fibers in Lambl's excrescences are shown (b). An intact endothelial cover was rarely observable on the surface of a Lambl's excrescence (c). The cross-shaped course of collagen fibers in Lambl's excrescences is shown (d).

also there were a lot of hollow spaces with interwoven layers of collagen fibers arranged in an orderly manner (Fig. 3b). In some sections it was seen that, besides only a few tangentially cut collagen fibers, on one side of the excrescences there was an intact endothelial cover, whereas on the other side there was none at all. Here the collagen fibers followed a cross-shaped course (Fig. 3c, d).

## Discussion

In this paper we present a systematic scanning and transmission electron microscopical study on explanted human aortic and mitral valves obtained from patients suffering from valvular heart disease. Our data show that there are several different alterations in the endothelium and the extracellular matrix, as judged by ultrastructural

investigations at high magnification. In all explanted specimens regardless of the affected valve type we found practically identical alterations throughout the structures of the valve. Morphological changes indicative of degenerative lesions were cellular swelling and loose binding of endothelial cells together with a loss of the endothelial layer over more or less widespread areas of the leaflets. Often endothelial ulcerations were accompanied by deposits of calcium-containing crystals. Normal endothelial cells were rarely identified at the surface of these lesions. The altered endothelial cells seemed to have reduced contact with each other. Sometimes the endothelium was missing over wide areas of the leaflet. Whether these loose connections between the endothelial cells and the cellular swelling finally result in the desquamation of the endothelial coverage is not clear.

Valvular masses, first described by Lambl more than a century ago, were reported in subsequent studies to occur predominantly in healthy subjects with a frequency of 90%.<sup>10,13</sup> From their growth patterns, two subtypes of excrescences, namely filiform and lamellar, were distinguished. The excrescences were observed in almost all specimens from patients older than 10 years and were located concomitantly in the nodular and subnodular regions and at the free margin of the leaflet. The nodular excrescences were present in 94% of the valves containing excrescences, whereas subnodular excrescences were present in 50% of the valves with excrescences, showing significant differences with age.<sup>10</sup> In valves from patients younger than 30 years, they were present in 78% of the leaflets with excrescences, whereas in those from patients older than 31 years they occurred in only 36%.<sup>10</sup> Excrescences at the free margin were observed in 22% of the valves with excrescences. They were not detected in specimens from patients younger than 37 years.<sup>10</sup> Lamellar excrescences displayed flattened structures up to 1.5 mm wide and 2 mm long, located preferentially in the subannular position.<sup>6</sup> The microscopical appearance of Lambl's excrescences is cell-poor, with fibrous connective and myofibrous tissue resembling histological aspects of papillary fibroelastomas.<sup>7,9,10,14</sup> Although the histological features of Lambl's excrescences and papillary fibroelastomas are virtually identical in some instances, both lesions are nosologically separated due to the gross appearances, the unusual locations and potential clinical sequelae of papillary fibroelastomas.<sup>14</sup> Lambl's excrescences are usually smaller and broader-based than papillary fibroelastomas.<sup>14</sup>

Interestingly, the ratio between the filiform and lamel-

lar form was approximately 80% in all samples. Excrescences at the free margin of the leaflet were observed in the majority of all specimens.<sup>10</sup> The appearance of excrescences at the edges of the leaflets and in areas of attachment of aortic or mitral rings suggested that mechanical stress may have induced these alterations. In accordance with results obtained from healthy subjects, the frequent presence of the excrescences at these sites may be indicative of a disturbed blood flow at the free margin of the leaflets. Thus, it is conceivable that the development of hyperplastic lesions is triggered by abnormal flow turbulences, and that these altered flow conditions may cause at least some of the clinical symptoms associated with these lesions.<sup>15,16</sup>

Our results demonstrate that Lambl's excrescences appearing in pathological aortic and mitral valves often displayed surface areas lacking an intact endothelial cover. This could be caused by the excessive synthesis of fibrous material at the base of the excrescences and the observed loose binding between the endothelial cells. These changes in the endothelium could, on the one hand, be due to the exposure of the basement membrane which itself sets a series of pathophysiological mechanisms in motion. On the other hand, the accumulation of hyperplastic extracellular matrix and in particular collagen fibers may indicate a dysfunction of fibroblastic cells leading to enhanced matrix synthesis.

In summary, in acquired aortic or mitral valve disease both degenerative and hyperplastic alterations can be seen. Since the explanted aortic and mitral valves were harvested in the final stage of disease when it was well advanced, we cannot evaluate the time course of structural alterations in the affected valves. The described valvular lesions are obviously the final stage of a highly complex pathophysiological process. Further studies on less seriously pathologically altered valvular tissue will shed more light on the pathophysiological mechanisms involved in these valve diseases.

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