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Work in progress report - Valves

Increased mRNA expression of decorin in the prolapsing posterior leaflet of the mitral valve☆

M.A. Radermecker[a,*], R. Limet[a], C.M. Lapiere[b], B. Nusgens[b]

[a]*Department of Cardiovascular and Thoracic Surgery, University Hospital of Liège, CHU Sart-Tilman, 4000 Liege, Belgium*
[b]*Laboratory of Connective Tissues Biology, University Hospital of Liège, Liege, Belgium*

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Abstract

To improve our understanding of myxomatous degeneration of the valvar tissue as seen in mitral valve prolapse, we have compared the biosynthetic phenotype of the connective tissue cells in myxomatous segments ($n = 4$) resected during surgery with that of homologous segments of normal valves ($n = 4$) harvested in age-matched organ donors. The steady-state level of mRNA for selected extracellular matrix macromolecules and metalloproteinases was assessed by quantitative (internal standard controlled) reverse transcriptase-polymerase chain reaction (RT-PCR). Among the investigated gene products, the decorin mRNA expression was significantly increased in degenerative valve compared with normal tissue (211 ± 48 vs. 100 ± 70, $p < 0.02$). The level of fibrillin 2 also tended to be increased (194 ± 88 vs. 100 ± 81, $p = 0.08$). These results suggest that myxomatous valvar tissue is characterized by an overexpression of mRNA for decorin. Owing to the role of this small leucine-rich proteoglycan in the regulation of fibril assembly and stability, this alteration may account for or is a result of a defective organization of the collagen and elastic fibers in this disease and contribute to the intrinsic distensibility and fragility of the myxomatous tissue.

Keywords: Mitral valve prolapse; Myxomatous degeneration; Collagen; Elastin; Fibrillin; Decorin; Metalloproteinase

1. Introduction

The mitral valve is a complex multilayered tissue created by specialized, functionally adapted cells. This tissue is exposed to permanent mechanical constraints, which requires the accommodation of changes in pressure regimen, shape and size throughout the cardiac cycle. Interaction between the interstitial cells and their surrounding extracellular matrix (ECM) leading to phenotypic modifications of the valvar tissue structure have already been documented under experimental conditions such as pressure [1] or volume overload [2]. Idiopathic mitral valve prolapse (MVP) is a frequent cause of mitral insufficiency (MI) in industrialized countries and results in a progressive MI in 15% of patients, over a 10–15-year period. Degenerative valves characterized by excess tissue of both leaflets with billowing and prolapse (Barlow disease) are clinically distinguished from other degenerative valves where, most often, only the median scallop of the posterior leaflet is involved. It has recently been shown, however, that these are two fairly distinct entities at the extremes of the spectrum of degenerative valve disease with only modest differences in qualitative histology [3].

The myxomatous aspects of the valve and the frequent association with inherited connective tissue disorders (Marfan, Ehlers–Danlos, pseudoxanthoma elasticum, etc.) suggest that an alteration of some components of the connective tissue may be involved [4]. Screening of families with MVP has suggested an autosomal dominant inheritance of that trait with age and sex-dependent expression. Recently, a first locus for myxomatous MVP (Barlow disease) was mapped within a few centimorgans interval [5]. The disabled gene(s) remain(s), however, unidentified. To gain insight into the mechanism of myxomatous valve degeneration, we have investigated the biosynthetic phenotype of the interstitial cells in the myxomatous tissue by quantitative reverse transcriptase-polymerase chain reaction

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* Corresponding author. Tel.: +32-4-366-71-63; fax: +32-4-366-71-64.
E-mail address: mradermecker@chu.ulg.ac.be (M.A. Radermecker).

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(RT-PCR) for some representative matrix macromolecules and metalloproteinases.

2. Material and methods

The study was performed on four prolapsing P2 segments of the mitral valve (medial scallop of the posterior leaflet) resected in a quadrangular fashion during mitral valvuloplasty (patient's mean age 51 ± 12 years). The morphological aspect was that of fibroelastic deficiency in the four patients with isolated prolapse of the median scallop of the posterior leaflet. Homologous segments of four normal valves were harvested in living multiorgan donors (mean age 49 ± 7) free from any valvular disease as judged from preoperative echo and morphological study. The specimens were kept frozen in liquid nitrogen until use or fixed for histological analysis (hematoxylin–eosine and orceine). This study was approved by the institutional ethics committee.

2.1. RNA extraction and RT-PCR conditions

Complete strips of the mitral valve (from annulus to free edge) were used for analysis to prevent the problem of valvar heterogeneity. The RT-PCR reactions were performed on purified total RNA in an automated thermal cycler (GeneAmp PCR System 2400 or 9600, Perkin Elmer, Norwalk, CT) using the GeneAmp Thermostable rtTh Reverse Transcriptase RNA PCR kit (Perkin Elmer) following previously described conditions and methods [6,7].

2.2. Expression of the results

The optical density (OD) of the amplification product of the selected mRNA was normalized to the OD of its own internal standard and expressed in arbitrary units per unit of 28S ribosomal RNA (28S rRNA) measured on a similar aliquot of the total RNA solution. To obtain comparative data for each specific mRNA, the whole series of normal and pathological samples was analyzed simultaneously in triplicate in the same run. The results in Table 1 are expressed as mean values ± standard deviation normalized to 100 for the control sample. The statistical analyses were performed using Student's *t*-test.

Table 1
Steady state level of mRNAs for extracellular matrix macromolecules and metalloproteinases

	Number cycles	CTL	MMV	*t*-test (*p* value)
α1 I	23	100 ± 29	88 ± 20	NS
α1 III	23	100 ± 43	107 ± 23	NS
Elastin	35	100 ± 60	99 ± 28	NS
Fibrillin 1	26	100 ± 58	118 ± 30	NS
Fibrillin 2	34	100 ± 81	194 ± 88	0.08
Decorin	23	100 ± 70	211 ± 48	<0.02
MMP2	26	100 ± 25	97 ± 21	NS
MT1MMP	28	100 ± 42	115 ± 33	NS

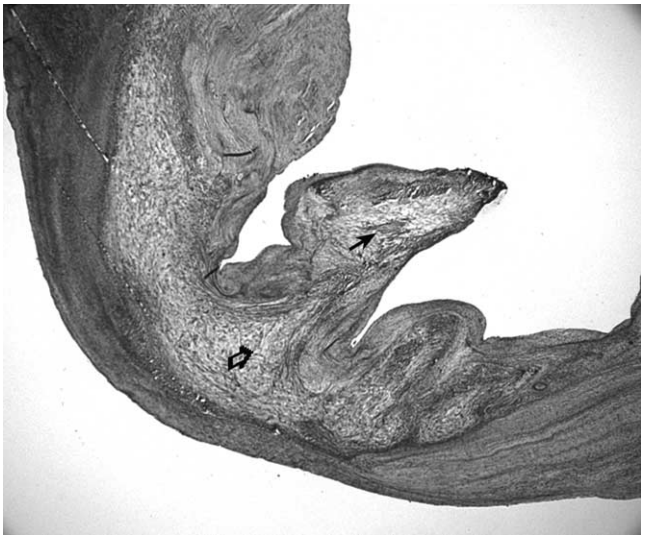

Fig. 1. Myxomatous degeneration of the posterior leaflet (orceine × 50). → Proliferation of the spongiosa; → infiltration of the chordae by the myxomatous process.

3. Results

3.1. Morphological analysis

Compared to the normal three layers arrangement, the anatomopathological hallmark of the myxomatous degeneration is a disorganization of the valvar tissue and threefold to fourfold increase in overall thickness of the leaflet (Fig. 1). Microscopically, myxomatous degeneration consists of infiltration of the valve by a hypertrophic spongiosa layer that focally invades and disrupts the fibrosa and auricularis. Continuation of the myxomatous process in the chordae tendinae was evidenced in the rough zone where the fibrosa merges into the chordae (Fig. 1). In the myxomatous valve, the auricularis showed an increased density of elastic fibers and fibrosis (Fig. 2).

The histological aspect of the four myxomatous valves was very similar in the texture of the matrix and cell density. The cell density in the spongiosa was 3.5-fold higher in the myxomatous, compared to the normal valves (75.2 ± 8.3 vs. 21.8 ± 9 cells per high power field). The mean relative proportion of each layer in the leaflet was also similar if examined from the free edge to the hinge area. The morphological aspect of the normal valves was very comparable in the four specimens.

3.2. Cellular phenotype

In this study, we investigated the expression of candidate genes potentially involved in myxomatous degeneration

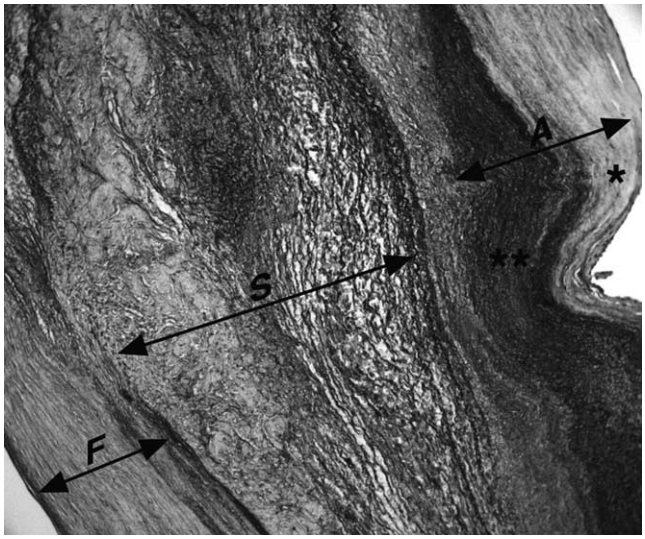

Fig. 2. Myxomatous valve (orceine × 200). A, auricularis; S, spongiosa; F, fibrosa. ∗ ∗ Increased density of elastic fibers in the auricularis layer. ∗ Fibrosis in the auricularis layer.

based upon morphological observations and/or their association with genetic disorders affecting the mitral valve, i.e. collagen types I and III, elastin, fibrillin 1 and 2, decorin and the constitutively expressed matrix metalloproteinases, MMP2 and MT1 MMP. In both Table 1 and Fig. 3, the level of the investigated mRNAs per unit of 28S rRNA have been normalized to the mean value of the controls (C) taken as 100 to illustrate the comparative analysis.

The investigated mRNAs are not expressed to the same extent. In Table 1, the number of cycles required to observe a measurable signal is provided for information. Collagen I and III and decorin, requiring less cycles of amplification, are the most abundant within the investigated mRNAs while elastin and fibrillin 2 are much less expressed. The individual values for each control specimen in Fig. 3 (each open symbol representing one patient) and for each myxomatous tissue (closed symbols) illustrate the variability of the mRNA expression. The range of variation of the individual values is remarkably narrow when considering the genetic heterogeneity of the human race except for decorin and fibrillin 2, which displays a somewhat higher variation than that of the other mRNAs. The mean values detailed in Table 1 demonstrate an overall similarity between C and MMV for most gene products except for the decorin mRNA level that is significantly higher in the pathological valves while that of fibrillin 2 tends also to be increased.

4. Discussion

The steady state level of mRNA expression per unit of 28S rRNA for collagen I and III is very similar in the normal and myxomatous tissues. An estimate of the relative number of copies of their mRNA has been calculated and gives a distribution of 46% type I—54% type III in the control valve and 40% type I—60% type III in the myxomatous samples. This difference, although not statistically significant ($p = 0.11$), might account for an increased proportion of collagen III in MMV. Indeed, mechanical stress has been reported to upregulate collagen synthesis in the mitral valve of experimental MI resulting from papillar muscle dysfunction [2]. Such a regulation is a common reaction of the connective tissue cells to increased tension [7]. The collagen network depends on a balance between synthesis and degradation and its ultimate and functional structure results from the assembly of collagen fibrils that can be modulated by various factors, among which minor collagens, degrading enzymes and proteoglycans [8]. Myxomatous tissue is characterized by an altered architectural organization of the collagen bundles, which are sparse, irregularly arranged and focally organized in spiraling substructures [4]. These abnormal polymers that retain their axial periodicity occupy a volume considerably larger than normal. Such abnormal organization and structure of the fibrils can be observed in diseases such as Marfan or Ehlers–Danlos type IV [11] in which an abnormal fibrillin 1 or defective collagen III, respectively, are likely to explain the weakness of the valve. It has been suggested that dysfunctions in enzymes responsible for the complex post-translational processing of procollagen could account for weakened extracellular matrix in the valve leading to prolapse. All the enzymatic deficiencies of this type are recessive trait making this hypothesis unlikely in the usual myxomatous degeneration that is autosomal dominant.

Semiquantitative immunohistochemical studies [9] have shown that myxomatous valves express increased levels of the interstitial collagenases MMP1 and MMP13 and the gelatinases MMP2 and MMP9. According to these studies, activation of MMPs is likely to play a role in the remodeling of the extracellular matrix, as seen in other diseases such as aneurysms, aortic valves in Marfan, etc. [10]. The levels of mRNA coding for the matrix metalloproteinases, MMP2 and its membrane-bound activator MT1MMP were similar in the control and myxomatous valves. No MMP3 mRNA was detected (data not shown), an enzyme also known to be induced by mechanical relaxation. A comparative screening of the expression of other proteinases is planned in the near future.

The increased level of decorin mRNA observed in our study suggests that this proteoglycan participates in the process. The small leucine-rich proteoglycans (lumican, biglycan and decorin) are involved in fibrillogenesis of collagen and elastin [12]. Decorin affects collagen types I and II fibrillogenesis in a core protein-dependent fashion by reducing the diameter of the collagen fibrils. Transgenic mice lacking decorin exhibit fragile skin and irregular collagen structures [13]. It has recently been shown that lumican and decorin have a different and distinct effect on fibril formation and independent binding sides on the collagen fibril. They seem to stabilize the fibrils at the same time as they limit their growth, preventing fibril

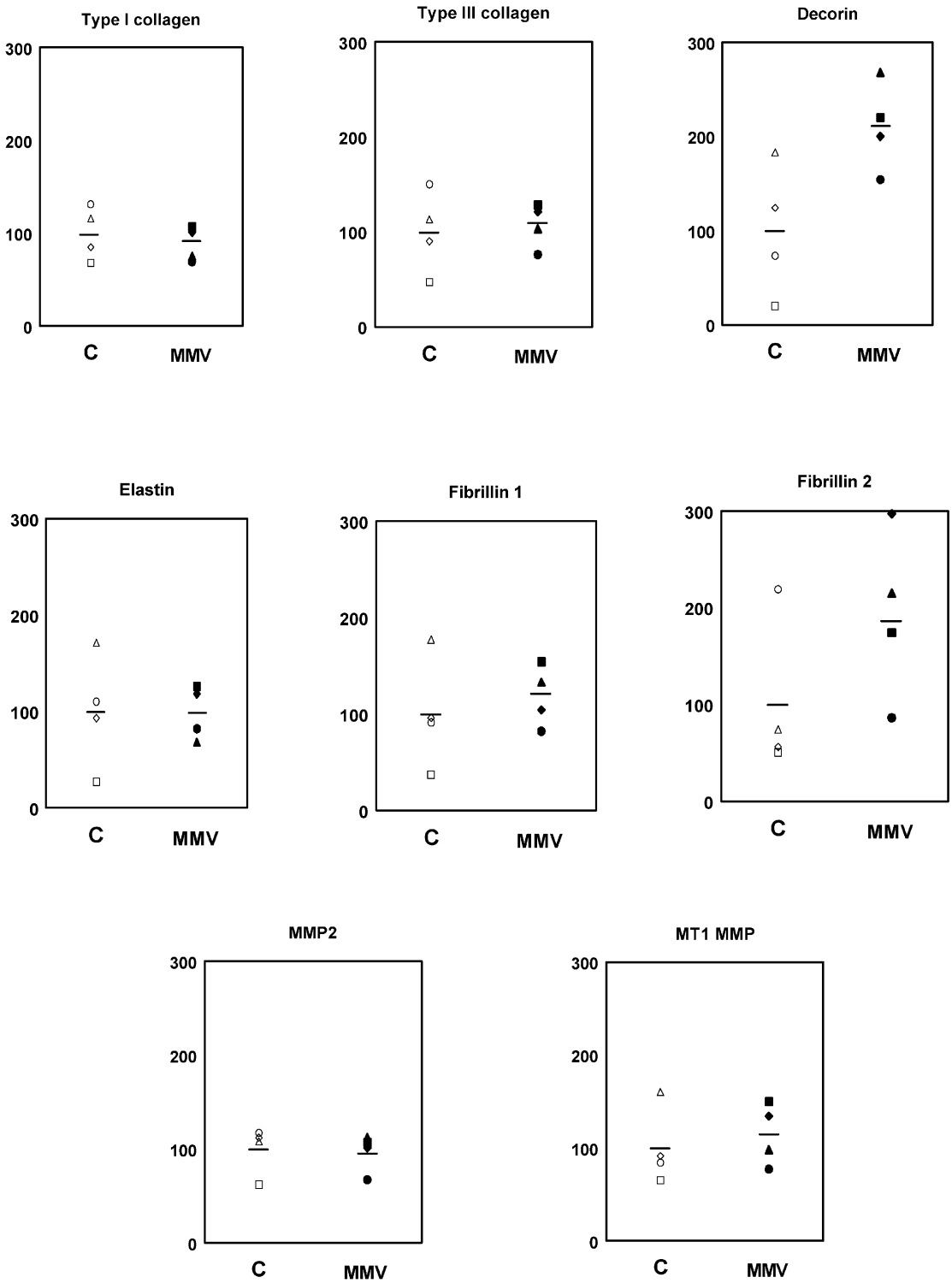

Fig. 3. Individual values of the mRNA levels measured in normal (open symbols) and myxomatous (closed symbols) valves. The results are normalized to the control values taken as 100. Each patient is represented by one individual symbol.

anastomosis and reducing their heterogeneity in diameter. Similarly, although not significant at the 5% level, the increased expression of fibrillin 2 is worth considering. This protein also associated in the elastic fibers has been claimed to regulate fibrillogenesis of the force bearing copolymers of elastin and fibrillin 1 [14]. We raise the possibility that an increased decorin production might represent a compensation mechanism related to the increased extracellular space created by defective fibrous polymers. We have observed such a process in the dermatosparactic skin containing disorganized fibers made of collagen precursors [15].

From our results and the literature, it can be proposed that myxomatous degeneration represents a final common pathway of valvar remodeling of potentially diverse etiology. It could be a result of exposition to abnormal stress as it might also depend on a defective ability of the cells to transduce the mechanical signals resulting from their interaction with the extracellular support. In the majority of cases, however, the primary deficiency is unknown, but the context points to genetic abnormalities, which result in an incapacity of the valve to bear or cope with normal stress. This may result from an abnormal morphology of the valve or mild defect at the cellular or molecular level. In this hypothesis, myxomatous degeneration should occur mainly in the median part of the posterior scallop, which is believed to be submitted to higher mechanical constraint. The activation of interstitial connective tissue cells in response to abnormal mechanical stress may appear as an adaptative response to enhance the capacity of the valvar tissue to sustain the mechanical constraint. Similar to what has been shown in cartilaginous tissue, where the proteoglycans are upregulated by increased pressure, the valvar spongiosa, which is believed to have a protective mechanical function by cushioning compressive stress and minimizing shear between fibrosa and auricularis is enhanced. If transiently adaptative, this response may lead to progressive alteration of the valvar tissue, possibly via the effect of an accumulation of proteoglycans among which decorin. Its increased concentration and its potential effect on fibrillogenesis of collagen and elastic fibers may perpetuate a vicious circle of reduced mechanical resistance by increasing the stress exerted on the valve, especially when mitral valve regurgitation occurs. In this scenario, repair surgery, although dealing with abnormal tissue may halt this process by reducing the undue stress on the leaflets and subvalvar structures. At the present time, it is not possible to determine if the overexpression of decorin is the cause or consequence of defective architectural organization of the fibrillar components of the extracellular matrix in myxomatous MVP. This is the subject of our current investigation.

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Appendix A. Conference discussion

Dr K. Zehr (*Rochester, MN, USA*): As you alluded earlier in your presentation, Barlow’s disease has often been thought of as a form fruste of Marfan syndrome which has been pinned down to the fibrillin 1 gene. Fleischer et al. from Hopkins years ago looked at Marfan tissue and found significant elastic disruption. I find it very interesting that fibrillin 1 was the same between your controls and your myxomatous patients.

Do you have access to Marfan P2 sections? We often fix the mitral valve regurgitation in the Marfan leaflets by doing P2 resections. Do you have access to that tissue and have you looked at these tissues to see if the changes that you found are present in Marfan tissue as well?

Dr Radermecker: No, we haven't, but we agree with you, and I think that most surgeons interested in the mitral valve concur by saying that myxomatous degeneration is just the final common pathway of a dysfunctioning leaflet, no matter the cause.

You pointed out Marfan with the problem of fibrillin 1, but Ehlers–Danlos type 4 also shows myxomatous floppy valves which are nearly the same as a Marfan valves, although the disorder is known in this case: it is the defect in the collagen type 3. This is just to point out that several known gene defects, fibrillin, type 3 collagen, may just lead to the same gross morphological disease, which is myxomatous degeneration.

That is why we are interested, even in form fruste of myxomatous mitral valve prolapse located only to P2, in understanding why someone let's say 45 years old with apparently normal connective tissue everywhere else, has a dysfunctioning P2 and a failure of the connective tissue to support his valve in this particular area. We believe that discrete gene defects or defective signaling between the cells and the extracellular matrix may be involved, and it is in that direction that we are currently pursuing our research.

Dr A. El-Ghafary (*Cairo, Egypt*): I think Dr Barlow from South Africa has found that 25% of the population has got mitral valve prolapse but are still asymptomatic, number one.

Number two, he did mention that one of the main causes of mitral valve prolapse probably in his group in South Africa is due to rheumatic origin. That is number two. So there is a big discrepancy between collagen which could not be seen as abnormal fibrillin and those patients who have got mitral valve prolapse and have got underlying heart disease like rheumatic heart disease in origin.

Did you correlate between the symptoms of the patients prior to surgery and the clinical data, because some of them have got severe arrhythmia of their prolapse, the second have got chest pain, and the third group have got also loss of consciousness? So did you correlate the abnormality that you have discovered in relation to the clinical data of those patients prior to the surgery or not?

Dr Radermecker: We don't have a follow-up of these patients, at least they haven't been followed so long before the operation so that we cannot tell you anything about the evolution of their disease. Concerning your previous remark, there is no possibility that degenerative mitral valve disease has been confounded with rheumatic etiology in this study. We agree with you that mitral valve prolapse is very frequent in the population, as published by Procacci et al. However, there is constant confusion between what I would call myxomatous mitral valve prolapse limited to P2 and the billowing and prolapsing valve which is the characteristic of Barlow's disease. These are two visible aspects of a disease at the extremes of a continuum. The disease is actually degenerative mitral valve disease because we have no better way to call it at this point in time.

Mr B. Keogh (*Birmingham, UK*): May I simply make an observation with respect to your diagram. I think in medicine it is becoming increasingly clear that very, very few diseases, particularly those with a variable phenotype, are due to a single gene defect, and that is no better illustrated, for example, than by hypotrophic obstructive cardiomyopathy.

Therefore, I find myself wondering whether your 'gene defect' should be in the plural and the question remains as to whether, in fact, the gene defect actually causes the morphological anomaly. Invariably when you see one morphological anomaly in the valve it looks as though similar problems are about to occur somewhere else and the issue of physical influences arises.

So you might start looking at valves where the morphological anomaly is due to something clearly physical, such as old, treated infective endocarditis, and if you start to see the subsequent interstitial cell changes there, then you know that morphological anomalies per se can create a problem. Otherwise I would suggest that the morphological anomaly should sit in between multiple gene defects and the rest of your boxes. It is just a thought.

Dr Radermecker: I agree, for the plural of 'gene defects'. And to add a little something, let me just add that maybe there is no gene defect but just a problem of regulation of functioning genes. So the situation is quite complex and this accounts for what we find in the clinical situation. There is a spectrum of degenerative valves.

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