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REVIEW ARTICLE

Degenerative valve disease and bioprostheses: risk assessment, predictive diagnosis, personalised treatments

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Abstract Aortic stenosis (AS) is the most frequent valvular heart disease. Severe AS results in concentric left ventricular hypertrophy, and ultimately, the heart dilates and fails. During a long period of time patients remain asymptomatic. In this period a pathology progression should be monitored and effectively thwarted by targeted measures. A cascade of cellular and molecular events leads to chronic degeneration of aortic valves. There are some molecular attributes characteristic for the process of valvular degeneration with clear functional link between shifted cell-cycle control, calcification and tissue remodelling of aortic valves. Bioactivity of implanted bioprosthesis is assumed to result in its dysfunction. Age, gender (females), smoking, *Diabetes mellitus*, and high cholesterol level dramatically shorten the re-operation time. Therefore, predictive and preventive measures would be highly beneficial, in particular for young female diabetes-predisposed patients. Molecular signature of valvular degeneration is reviewed here with emphases on clinical meaning, risk-assessment, predictive diagnosis, individualised treatments.

Keywords Degenerative valve disease · Bioprostheses · Risk assessment · Diabetes · Predictive diagnosis · Personalised medicine

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Degenerative valve disease: clinical aspects and molecular signature

Clinical assessment of aortic stenosis

Aortic stenosis (AS) is the most frequent valvular heart disease. Its prevalence increases with age, and has been reported between 2–4% in a population ≥65 years old [1, 2]. Aortic sclerosis is the precursor of AS and has been found in 25–30% [3]. Calcific AS refers to a narrowing of the aortic valve lumen as a result of the deposition of calcium in the cusps and valve ring. Severe AS results in concentric left ventricular hypertrophy, and ultimately, the heart dilates and fails. During a long period with increasing outflow tract obstruction, which results in increasing left ventricular pressure load, patients remain asymptomatic, acute complications are rare. Therefore, these asymptomatic patients with AS should be monitored closely for the development of symptoms and progression of disease, especially by Doppler-echocardiography, an accurate non-invasive measurement of the stenosis severity (Fig. 1).

However, as soon as symptoms occur, such as exertional dyspnoea, angina, and syncope, outcome becomes poor. Average survival after the onset of symptoms has been reported to be less than 2–3 years [4]. In this situation, valve replacement does not only result in dramatic symptomatic improvement but also in good long term survival [5]. This holds true even for patients with already reduced left ventricular function, as long as functional impairment is, indeed, caused by AS. Thus, there is general

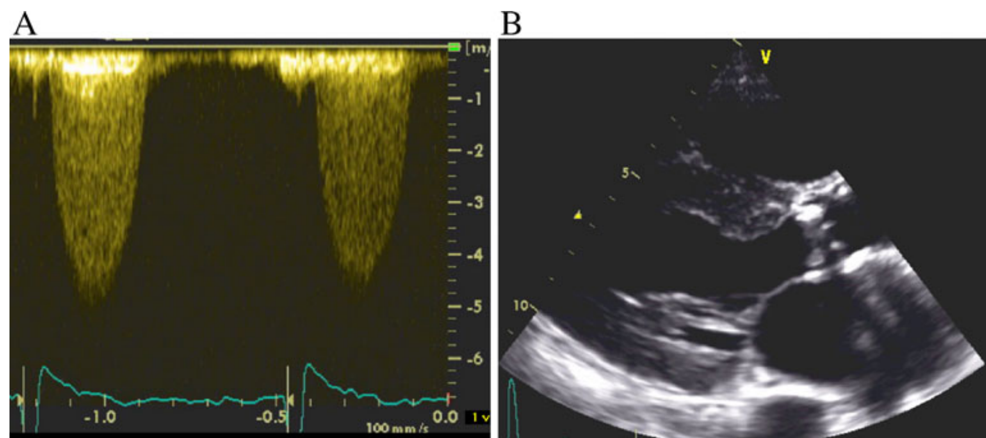

Fig. 1 Clinical assessment by (**a**) Doppler echocardiography; (**b**) two dimensional echocardiography (parasternal short axis view)

agreement that urgent surgery must be strongly recommended in symptomatic patients [5–7].

A cascade of cellular and molecular events leads to chronic degeneration of aortic valves

Mechanical stress is currently considered as the main cause that triggers degenerative processes. This is accompanied by a thickening of the valve cusps, and remodelling of the left ventricular geometry. Clinical-pathological studies of aortic stenosis have demonstrated an abundant deposition of extracellular matrix (ECM) proteins physiologically present in bones [6], and cuspal calcific deposits associated with mineralisation of devitalised cells [8]. Moreover, bone-marrow derived endothelial progenitor and dendritic cells have been identified in both native degenerative aortic valves and degenerative prostheses; the co-localisation of those cells with inflammatory infiltrates has been demonstrated [9]. A cascade of cellular and molecular events leading to the degeneration of aortic valves is summarised in Fig. 2.

Mineralisation of skeletal and dental tissue is genetically programmed and physiologically well-regulated. In contrast, non-physiological calcification occurs in numerous pathological cardiovascular conditions including atherosclerosis, valvular stenosis, and reperfused ischemic myocardium. This is proposed to be an undesired common feature of degenerative or / and inflammatory tissue changes throughout the body. Pathomechanisms leading to the calcification of heart valves are still largely unknown. Contrary to physiological formation of bones, cuspal calcific deposits in the heart are non-physiological and normally not found in healthy cardiovascular tissues [6, 8, 10–12]. Numerous clinical-pathological studies of calcified valves have demonstrated cuspal calcific deposits tightly associated with mineralisation of devitalised cells, indicating a cascade of (programmed?) molecular events leading to chronic degeneration of myocardial tissue [6]. Tissue homeostasis strictly depends on a balance between cell growth and death. These aspects have been investigated at the level of gene transcription as reported earlier [7]: Table 1 summarises the list of gene products, a corresponding function of which is suppressed specifically in calcified *versus* non-calcified aortic valves. Among them, 40 proteins essential for energy metabolism are suppressed by aortic calcification. Furthermore, an expression of cytoskeleton-formation as well as ECM-building and tissue remodelling proteins (altogether 23 proteins) is completely suppressed in calcified valvular tissue. The above given protein core is switched off specifically in the case when the balance between cell growth and death in tissue homeostasis is shifted towards cellular death.

Taking these data together, a well-coordinated programme of molecular events targeted in cellular death can be

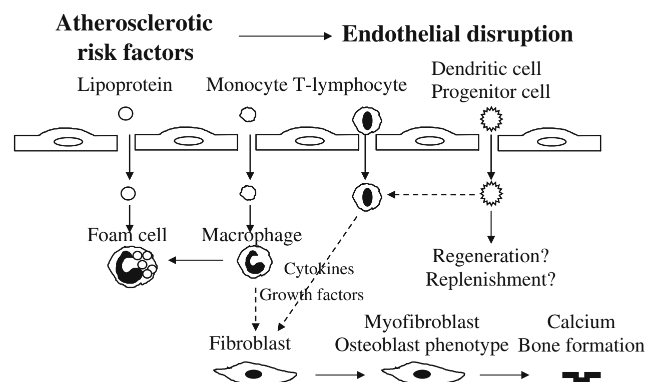

Fig. 2 Cascade of cellular and molecular events leading to the degeneration of aortic valves: Inflammatory cells release cytokines and growth factors that act on valve fibroblasts. A subset of myofibroblasts may differentiate into the osteoblast cell-phenotype that secretes bone matrix proteins involved in the valve calcification process. However, several question remain open, such as - whether there is a differential role of the multiple subset of immune / inflammatory cells in the depicted cascade of events followed by the question, - whether the above demonstrated cellular events can serve as indicators for predictive diagnostics at pre-stages of valvular calcification

Table 1 The data represent 63 gene products, the function of which is suppressed in calcified versus non-calcified degenerated aortic valves. There are following functional groups: energy metabolism, proteins responsible for cytoskeleton formation, matrix building, and tissue remodelling [7]

GeneBank Accession / SwissProt Accession		Gene (protein) name / function
I. Energy metabolism proteins (40 genes)		
S70154	Q16146	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)
D90228	P24752	acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)
L07033	P35914	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)
X83618	P54868	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)
U62961	P55809	3-oxoacid CoA transferase
M93107	Q02338	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)
X17025	Q13907	isopentenyl-diphosphate delta isomerase
X69141	P37268	farnesyl-diphosphate farnesyltransferase 1
M88468	Q03426	mevalonate kinase (mevalonic aciduria)
U49260	P53602	mevalonate (diphospho) decarboxylase
D78130	Q14534	squalene epoxidase
D63807	P48449 Q9UEZ1	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
AF034544	O60492	7-dehydrocholesterol reductase
U60205	Q15800	sterol-C4-methyl oxidase-like
M67466	P14060 Q14545 P26439	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2
Y09501	P00387	diaphorase (NADH) (cytochrome b-5 reductase)
L21934	P35610	sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1
R07932		diacylglycerol O-acyltransferase homolog 1 (mouse)
M74047	P31213	steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2)
L33179	Q13713 P40394	alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide
M68895	P28332	alcohol dehydrogenase 6 (class V)
M63967	P30837	aldehyde dehydrogenase 1 family, member B1
X05409	P05091 Q03639	aldehyde dehydrogenase 2 family (mitochondrial)
M73704	Q00169	phosphotidylinositol transfer protein
L34081	Q14032	bile acid Coenzyme A: amino acid N-acyltransferase (glycine N-choloyltransferase)
U47105	Q15738	NAD(P) dependent steroid dehydrogenase-like; H105e3
X05130	P30037 P32079 Q15205 P07237	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)
U12424	P43304	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
L34041	P21695	glycerol-3-phosphate dehydrogenase 1 (soluble)
D88308	O14975	fatty-acid-Coenzyme A ligase, very long-chain 1
L09229	P41215 P33121	fatty-acid-Coenzyme A ligase, long-chain 1
X83368	P48736	phosphoinositide-3-kinase, catalytic, gamma polypeptide
S67334	P42338	phosphoinositide-3-kinase, catalytic, beta polypeptide
X66922	P29218	inositol(myo)-1(or 4)-monophosphatase 1
M74161	P32019	inositol polyphosphate-5-phosphatase, 75kD
L08488	P49441	inositol polyphosphate-1-phosphatase
D16481	P55084	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit

Table 1 (continued)

GeneBank Accession / SwissProt Accession		Gene (protein) name / function
U40002	Q05469	lipase, hormone-sensitive
M72393	P47712	phospholipase A2, group IVA (cytosolic, calcium-dependent)
U20157	Q15692 Q13093	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
II. Cytoskeleton formation, ECM-building & tissue-remodelling proteins (23 genes)		
X58141	P35611	adducin 1 (alpha)
X58199	P35612	adducin 2 (beta)
M58018	P12883 Q14904 Q16579	myosin, heavy polypeptide 7, cardiac muscle, beta
M63603	P26678	Phospholamban
X92762	Q16635	tafazzin (cardiomyopathy, dilated 3A (X-linked); endocardial fibroelastosis 2; Barth syndrome)
X56134	P08670	vimentin
J03209	P08254 P09238	matrix metalloproteinase 3 (stromelysin 1, progelatinase)
D83646	P51512	matrix metalloproteinase 16 (membrane-inserted)
X75308	P45452	matrix metalloproteinase 13 (collagenase 3)
X07819	P09237	matrix metalloproteinase 7 (matrilysin, uterine)
J05070	P14780	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
X89576	Q14850	matrix metalloproteinase 17 (membrane-inserted)
L23808	P39900	matrix metalloproteinase 12 (macrophage elastase)
J05556	P22894	matrix metalloproteinase 8 (neutrophil collagenase)
J03210	P08253	matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase)
X57766	P24347	matrix metalloproteinase 11 (stromelysin 3)
X03124	P01033 Q14252	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)
U14394	P35625	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
U76456	Q99727	tissue inhibitor of metalloproteinase 4
L00073	P00797	renin
J04144	P12821	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
L13977	P42785	prolylcarboxypeptidase (angiotensinase C)
K02566	P01043	kininogen

postulated considering the pathomechanisms of aortic valve calcification. However, before the end-point is reached when valve tissue is calcified, a long-time chronic process of degeneration occurs in the valve tissue.

Molecular attributes characteristic for the process of valvular degeneration

Altogether 99 genes have been reported earlier with the expression well detectable in calcified aortic valves (Table 2, [7]). Thereby, an expression level of 42 genes remains unaffected by the grade (calcified versus non-calcified) of degeneration severity such as albumin, specific receptors of oxidised low-density lipoprotein, advanced glycosylation end-products and natriuretic-peptide, potassium inwardly-rectifying channel-5, gap-junction proteins, particular integrins, tropins and cadherins [7]. However, the majority (57 proteins) detected was highly affected as a function of the degeneration grade: these are potassium voltage-gated channel-1, cardiotrophin, cardiac myosins, metalloproteinases, endothelins, neuropilins, caveolins, progesterone-, vasopressin-, tumour-necrosis-factor- and adrenergic-receptors. Moreover, whereas well-expressed hepatic lipase has been demonstrated in calcified valves, no traces of its expression could be detected in non-calcified tissue. Those gene products should be taken into account as the stage-specific targets in the cascade of cellular and molecular events that accompany chronic aortic degeneration for a predictive diagnosis and considering individualised therapeutic approaches.

Table 2 Among 99 gene reported to be expressed at the transcriptional level in human calcified degenerated aortic valves, there are 57 gene products listed below the expression level of which is specifically altered as compared to non-calcified valves [7]

GeneBank Accession / SwissProt Accession		Gene (protein) name / function
Increased		
M65199	P20800	endothelin 2
L25615	P37288	arginine vasopressin receptor 1A
Z11687	P30518	arginine vasopressin receptor 2 (nephrogenic diabetes insipidus)
D31833	P47901	arginine vasopressin receptor 1B
L02911	Q04771	activin A receptor, type I
AF015257	Q99527 Q99981 O00143 Q13631	G protein-coupled receptor 30
L35545	Q14218	protein C receptor, endothelial (EPCR)
AJ002962	O15540 O14951	fatty acid binding protein 7, brain
M86917	P22059	oxysterol binding protein
L06133	Q04656	ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)
U50743	P54710	FXYD domain-containing ion transport regulator 2
U89364	P51787 Q92960	potassium voltage-gated channel, KQT-like subfamily, member 1
M93718	P29474	nitric oxide synthase 3 (endothelial cell)
U05291	Q06828 Q15331	fibromodulin
S73813	P49961	ectonucleoside triphosphate diphosphohydrolase 1
M90657	P30408	transmembrane 4 superfamily member 1
D26512	P50281	matrix metalloproteinase 14 (membrane-inserted)
S39329	P20151	kallikrein 2, prostatic
M13143	P03952	kallikrein B, plasma (Fletcher factor) 1
J05262	P14324	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)
X68505	Q02078 Q14223 Q14224	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)
X07228	P78529 P11150	lipase, hepatic
Decreased		
M21121	P13501 O43646	small inducible cytokine A5 (RANTES)
M31210	P21453	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1
U03865	P35368	adrenergic, alpha-1B-, receptor
AF016098	O60462	neuropilin 2
AF016050	O14786 O60461	neuropilin 1
U41070	Q15722 Q13305 Q92641	leukotriene b4 receptor (chemokine receptor-like 1)
U01839	Q16570 Q16300	Duffy blood group
Y12711	O00264	progesterone receptor membrane component 1
L49399	Q13772	nuclear receptor coactivator 4
J04739	P17213	bactericidal/permeability-increasing protein
L27213	P48751	solute carrier family 4, anion exchanger, member 3

Table 2 (continued)

GeneBank Accession / SwissProt Accession		Gene (protein) name / function
M20747	P14672	solute carrier family 2 (facilitated glucose transporter), member 4
X52882	P17987 Q15556	t-complex 1
Z18951	Q03135	caveolin 1, caveolae protein, 22kD
AF035752	P51636	caveolin 2
AF043101	P56539	caveolin 3
X60592	P25942	tumor necrosis factor receptor superfamily, member 5
AB000895	O15098	protocadherin 16 dachsous-like (Drosophila)
AF047826	O60574	cadherin 19, type 2
AF016272	P75309	cadherin 16, KSP-cadherin
AB006757	O60247	BH-protocadherin (brain-heart)
L34954	P36382	gap junction protein, alpha 5, 40kD (connexin 40)
X87241	Q14517	FAT tumor suppressor homolog 1 (Drosophila)
M14993	P11171	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)
U49837	P50461	cysteine and glycine-rich protein 3 (cardiac LIM protein)
U43030	Q16619	cardiotrophin 1
M94547	Q01449	myosin light chain 2a
X84075	Q14896	myosin binding protein C, cardiac
D00943	P13533 Q13943 Q14906	myosin, heavy polypeptide 6, cardiac muscle, alpha (cardiomyopathy, hypertrophic 1)
M86406	P35609	actinin, alpha 2
U02031	Q12772	sterol regulatory element binding transcription factor 2
L10413	P49354	farnesyltransferase, CAAX box, alpha
Y08200	Q92696	Rab geranylgeranyltransferase, alpha subunit
Y12856	O00286	protein kinase, AMP-activated, alpha 1 catalytic subunit
U16660	Q13011	enoyl Coenzyme A hydratase 1, peroxisomal

A functional link between cell cycle-control and calcification of aortic valves: potential diagnostic and prognostic targets

A proper control over cell-cycle progression seems to be a crucial step in the maintenance of a physiological cell population. Although cardiac cells undergo terminal differentiation soon after birth, irreversibly withdrawing from the cell-cycle, growth stimulation induces cell hypertrophy, the first visible step of a developing imbalance in the maintenance of the cardiac cell population. The hypertrophic growth has been shown to be associated with the re-activation of the fetal gene programme in cardiac cells – the key event is the positive regulation of a cell-cycle progression [13–15]. This switch in the programme seems to be crucial for myocardial cell regulation. Such growth stimulation is responsible for the up-regulated activity of cyclin-dependent kinases, CDKs, that consist of a kinase-core and an associated cyclin-subunit acting as the positive regulator [16]. In the matter, different CDK inhibitors keep a negative control over CDK activities. CDK inhibitors are classified on the basis of their sequence homology and substrate specificity. A cardiac helicase CHAMP was described as inhibiting cell proliferation and cardiac hypertrophy [13]. The CHAMP-dependent inhibition of cardiac hypertrophy is accompanied by the strictly programmed up-regulation of the cyclin-dependent protein-kinase inhibitor $P21^{WAF1/CIP1}$, a 21-kDa protein and member of the CIP/KIP family [16]. Furthermore, the targeted over-expression of $P21^{WAF1/CIP1}$ prevents cell enlargement and suppresses a specific gene expression of cardiac hypertrophy markers in the cell population in vitro [17] indicating the key role of $p21^{WAF1/CIP1}$ in the regulation of the hypertrophic response.

The physiological expression of $p21^{WAF1/CIP1}$ shows a gradual increase during development in both rat and man, becoming maximal in adulthood [18]. A direct link between the Bcl-2 dependent down-regulation of $p21^{WAF1/CIP1}$ and an increased myocyte density in the left ventricle has been shown in experimental work with transgenic mice [19]. These findings are in agreement with those achieved by

examination of human tissue: the coordinated down-regulation of both G_1 and G_2 checkpoint genes p21$^{WAF1/CIP1}$ and 14-3-3-sigma, respectively, correlates well with increasing cardiac cell density and the calcification appearance of aortic valve tissue [20]. The coordinated suppression of checkpoint genes in calcified aortic valves at both transcription (A) and translation (B) levels is represented in Fig. 3 [21]. Both cellularity and number of macrophages are significantly increased in calcified tissue (see Fig. 3c, d, respectively) [21]. According to the monitored CD68 positive signals, macrophages are localised predominantly in the sub-endothelial layer of the valvular fibrosa, whereas 14-3-3-sigma and p21$^{WAF1/CIP1}$ can be observed in both sub-endothelial layer and valvular interstitium of non-calcified tissue, being mainly co-localised with alpha-actin in the valvular spongiosa and pointing to the target expression in myofibroblasts. There is a growing body of evidence that in response to stimulus/injury the heart valves undergo tissue remodelling including phenotypic modulation and transformation of fibroblast-like into myofibroblast-like cells [22]. Therefore, the target protein expression of 14-3-3-sigma and p21$^{WAF1/CIP1}$ observed in degenerated valvular tissue, can originate predominantly from myofibroblasts.

Moreover, both the increased cell density and coordinated down-regulation of p21$^{WAF1/CIP1}$ and 14-3-3-sigma gene expression were found to be characteristic for calcification, in contrast to non-calcified valvular tissue [23]. Therefore, the double-control via both check-point proteins over DNA quality and cell proliferation in valvular cells might be efficient only in non-calcified tissue, whereas in the calcifying one this function is getting suppressed at both G_1 and G_2 phases of cell-cycle. These findings give further evidence that the efficiency of cell-cycle control in human non-calcified valvular tissue depends not only on the positive/negative CDK regulation in the G_1 phase but also on the coordinated regulation of both G_1 and G_2 dependent checkpoints. Further in vitro experiments on rat cardiac fibroblasts showed that a target up-regulation of inhibitors for G_1 dependent CDKs effectively suppresses the DNA synthesis and may decrease a potential risk of cardiovascular diseases [23].

The dissociation of P21$^{WAF1/CIP1}$ from the CDK complexes correlates well with the activation of CDK2, CDK4, CDK6, and the release from cell-cycle arrest, whereby the number of cardiac cells in S phase rises considerably [24]. Further, in contrast to P16 (a specific inhibitor of CDK4/6), the "universal" CDK inhibitor P21$^{WAF1/CIP1}$ was shown to be able to block completely an E2F-1-induced G_1 exit [25]. However, E1A binding activity to target protein complexes has effects on the cell-cycle progression beyond those produced by E2F-1 alone and can drive S-phase entry that is resistant to P21$^{WAF1/CIP1}$ [24]. These facts explain the necessity of the coordinated regulation of both G_1 and G_2 dependent checkpoints, in order to keep the control over the cell population maintenance in cardiac tissue.

Pronounced up-regulation of both genes in non-calcified in contrast to their down-regulation in calcified degenerated valvular tissue indicates the central regulatory role of checkpoint genes in keeping functional the valvular cells.

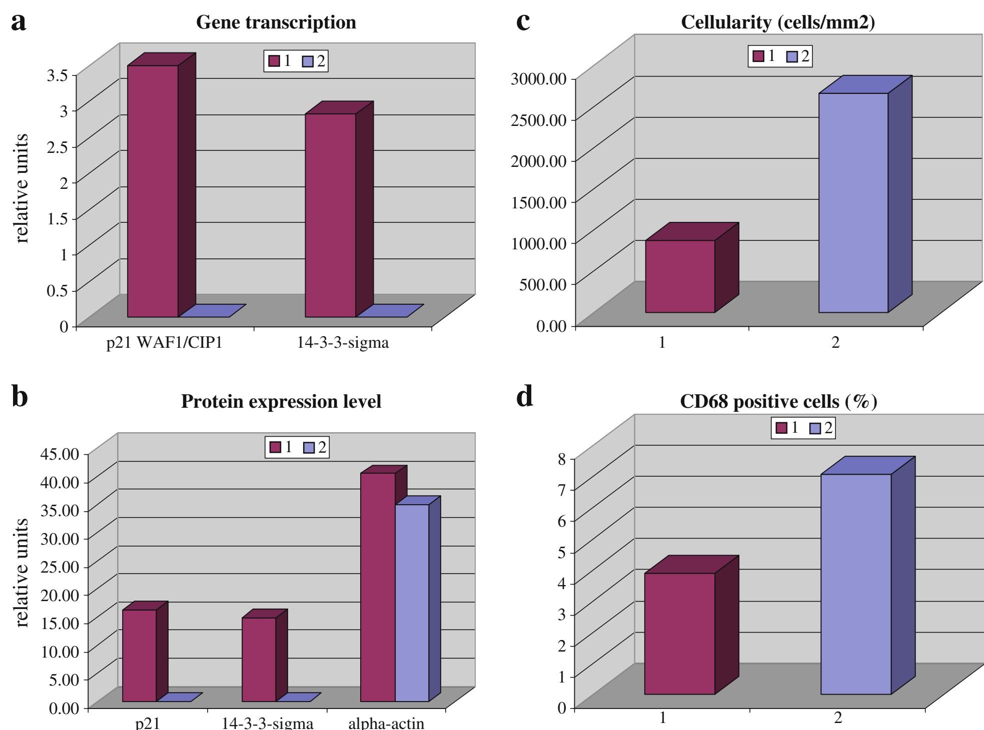

Fig. 3 Comparative analysis in two groups of patients with non-calcified (1) versus calcified (2) degenerative aortic valves. All analyses have been performed as described earlier [20]. The corresponding mean values are presented with statistically significant differences between the groups of comparison. (**a**) Comparative gene expression analysis (mRNA level in relative units) of p21$^{WAF1/CIP1}$ and 14-3-3-sigma. Quantitative Real-Time-PCR was applied. Beta-actin was used as the house-keeping gene for normalisation of corresponding values of the target gene expression rates. (**b**) Comparative analysis of protein expression levels (in relative units) of P21$^{WAF1/CIP1}$, 14-3-3-sigma and alpha-actin. (**c**) Comparative analysis of cellular density (in relative units). (**d**) Comparative analysis of macrophages (in relative units)

Blockade of cell-cycle progression results in a prolonged resistance to macrophage invasion and foam cell deposition [26]. Therefore, it is likely that reduced cell-cycle control in valvular tissue leads to the increased macrophage invasion that, in turn, can contribute to non-physiological calcification by both triggered unspecific inflammation and NO-toxicity [27–31]. Taken together, the coordinated activation of both G_1 and G_2 dependent checkpoint genes may be an attribute of the valvular tissue resistance against the calcification processes. These data should be taken into consideration to design novel therapeutic approaches targeted at pro-calcification mechanisms in the heart.

Risk assessment: factors involved in degenerative valve disease

Recent studies demonstrate an association between atherosclerosis and AS. Traditional cardiovascular risk factors such as lipid disorders, diabetes, arterial hypertension, smoking and male gender [32, 33] are reported to increase also the incidence of AS. At least one of these factors or, more frequently, even the combination of them is usually observed in this cohort of patients [20]. Although advanced age is the main risk factor, worldwide statistics indicate that degenerative aortic valve disease (DAVD) cannot be explained by ageing alone. No longer considered as a natural consequence of ageing, DAVD is the result of actively driven pathological processes including programmed (de)regulation of target genes, metabolic alterations, inflammatory cell infiltration, subcellular disruption, and consequent tissue degeneration, calcification and remodelling [20]. Due to extremely high morbidity and mortality caused by DAVD particularly in Western world, the central question has to be answered: Is an individual predisposition to the disease predictable? From this viewpoint a clear definition of disease specific risk factors is of particular interest.

Although the causal mechanisms are still largely unclear, all molecular as well as cellular processes attributed to DAVD are generally triggered secondarily to a central metabolic failure (diabetes, hypercholesterolemia, hypercalcaemia, leanness), hormonal deregulation (hyperparathyroidism), hypertension, and extreme stress conditions such as tobacco use and environmental stress factors [34–37]. Thus, an inverse relationship was demonstrated between body mass index and DAVD incidence: calcific changes were more frequently observed in lean people even independently of the risk factor of age, and, therefore, cannot be explained by leanness frequently observed in patients with highly advanced age. These facts indicate, further, an association of DAVD with metabolic disorders causing weight loss such as osteoporosis [36].

In diabetes, an increased production of highly aggressive reactive oxygen species (ROS) under hyperglycaemic conditions is considered as the main trigger for severe, chronic complications such as DAVD. Moreover, using advanced biomedical technologies such as clinical proteomics, individual stress reactions and resulting complications can be quite precisely predicted; disease specific molecular markers are already close to their clinical application specifically for the diabetic complication [38]. Similarly to diabetic patients, smokers also suffer from highly increased ROS production leading to enhanced incidence of DAVD, although specific pathomechanisms deserve further clarification. Deregulation of angiotensin-II metabolism and activity of angiotensin-specific receptors is considered to be the key molecule in the pathomechanisms that underlie DAVD in hypertension [37, 39, 40].

Individualised treatment of aortic stenosis and prognosis

A large body of evidence indicates that aortic stenosis is an active process with a distinctive histological appearance, associated clinical factors, and, variable disease progression proposing that this disease may be amenable in terms of the variety of risk factors but also successful treatments by individualised therapeutic approaches to prevent or at least slow down the disease progression [41, 42]. Indeed, several retrospective studies have consistently demonstrated that statin-based treatments are associated with notably lower haemodynamic progression of aortic stenosis [43–46]; however, statins failed in the prospective SALTIRE trial. It was suggested that the beneficial effects by statin are independent of lowering cholesterol impacts [43, 44]. Interestingly, both CRP expression at the valvular tissue level and serum CRP levels were found to be significantly lower under statin-based treatments [47] suggesting its pleiotropic and/or anti-inflammatory properties. As demonstrated by several independent studies (SALTIRE, SEAS, ASTRONOMER) lowering LDL-cholesterol levels do not halt the progression of aortic stenosis in patients with mild to moderate aortic-valve disease [48, 49]. The fact that angiotensin converting enzyme (ACE) and angiotensin II can be found in sclerotic but not in normal aortic valves indicates an important role of the renin-angiotensin system (RAS) in the pathogenesis of AS [50]. Further, the RAS has already been shown to play an important role in atherosclerosis. Consequently, ACE inhibitors slow down the calcium accumulation in aortic valves [43]. However, studies evaluating the effects of ACE inhibitors [46] and angiotensin II type 1 receptor blockers [51] did not find any difference in haemodynamic progression of AS in untreated patients versus patients who were taking these drugs.

In conclusion, it is too early for recommendations in terms of prevention of AS progression by currently applied treatments: further studies are highly desired. The recommended approach to treat the symptomatic, advanced AS remains the prosthetic valve replacement. Moreover, there

is a clear consensus that urgent valve replacement is required for symptomatic AS, while the management of asymptomatic patients with severe AS is still controversially discussed. In the matter, inhibitors of angiotensin-converting enzyme are currently under extensive consideration for their therapeutic application to effectively prevent both hypertension and DAVD [37, 39, 43, 52]. Independently from individual risk factors, the crucial role of metalloproteinases in the central pathomechanisms of the progressive tissue remodelling during the chronic development of DAVD is well recognised [20, 53]. Novel therapeutic interventions consider, therefore, metalloproteinases as the preferred target to delay or even prevent the progression of DAVD [37].

Aortic valve replacement: risk factors, geometry remodelling, complications

Dysfunction and bioactivity of implanted bioprostheses

Twenty percent to thirty percent of implanted bioprostheses show dysfunction after about 10 years post-implantation. Recent reports predict that a greater than 50% incidence of failure will be seen in bioprostheses at 12–15 years [54]. In addition, risk factors of atherosclerosis as well as chronic renal disease and parathyroid tumours might play a substantial role in the degeneration of bioprostheses. In order to improve the quality of life after cardiac valvular surgery, innovative procedures and new generations of prostheses have been developed in the past decade. The most frequently used porcine bioprostheses have been demonstrated to be bioactive in the human organism. DNA and RNA analysis of non-implanted bioprostheses before aortic valve replacement (AVR) has revealed sequences able to hybridise to as many as 112 human genes/transcripts relevant to cardiovascular pathologies [7]. Among those genes there are several overlapping sequences, the expression of which strictly depends on the grade of degeneration: endothelins, sodium / calcium exchangers, potassium voltage-gated channel-1, metalloproteinases, vasopressin- and adrenergic-receptors. Altogether, there are 74 genes found to be specifically altered by expression in human calcified degenerated aortic valves as summarised in Table 3.

Currently, poor information is available concerning the bioactivity of prosthetic material when they are implanted in human valves. In vivo-hybridisation to human nucleic acids might be one feasible reason for several well-known complications triggered by implantation. Thus, worldwide statistics indicate that each kind of AVR is not rarely followed by different metabolic impairments and physiological complications such as progressively abnormal lipid profiles, a non-specific inflammation, blood trauma, haemorheologic changes or severe congestive heart failure and even death during individually long postoperative time [55–61]. After AVR, the wall thickness becomes significantly greater than normal for patients with aortic stenosis, and after 5 years of follow-up the remodelling of the left ventricular geometry is usually observed after AVR [62].

Tissue remodelling of replaced valves: matrix metalloproteinses as biomarkers and potential therapeutic targets

Matrix metalloproteinases (MMPs) play the key role in tissue remodelling under both physiological and pathological conditions. MMPs are produced as zymogens (pro-MMPs) that require proteolytic activation through the elimination of the N-terminal propeptide via membrane type-matrix metalloproteinase (MT-MMPs) activity. Tissue inhibitors of metalloproteinases (TIMPs) act to inhibit metalloproteinase activity by forming a non-covalent irreversible complex with MMPs. A shifted balance in resulting MMPs / TIMPs activity is well documented under stress conditions [58].

However, less is known about a regulation of ECM degrading enzymes in native degenerating aortic valves and in valvular tissue after replacement. Aortic valves tissue is characterised by considerable heterogeneity of the cellular population: endocardial, interstitial, smooth muscle cells as well as fibroblasts and myofibroblasts have been identified in highly sophisticated dynamic structures of cardiac valves [63]. The ECM is thought to be an integral component of this coordinated dynamism [64]. The cores of activated ECM degrading genes differ both qualitatively and quantitatively at each stage of valvular degeneration; after AVR it is regulated in a different manner [36]. The activation grade of the MMP cores is found to be specific for each stages of the valve degeneration: whereas MMP-9 activation differs quantitatively, an activation of MMP-2 was observed solely at the earliest stages of degenerative process [53, 65]. In contrast, the stage of progressive calcification is characterised by dropping of the ECM-degradation potential. Therefore, the highly activated ECM-degradation potential might be considered as an early marker for the triggered degeneration of valvular tissue. Consequently, ex vivo evaluation of the dynamic in the ECM-degradation potential, e.g. measured by comparative zymography in blood samples, seems to be of great prognostic value [66].

This is of note that the set-up of ECM-degrading enzymatic-core changes dramatically after AVR: in contrast to the expression rates well-detectable in native valvular tissue, neither MMP-2 expression nor this of MMP-9 was detected in the replaced tissue. In addition, TIMP-1 was shown to be activated in the valves after replacement. TIMP-1 represents the very last step in the negative

Table 3 DNA and RNA analysis of porcine bioprosthetic material before the aortic valve replacement revealed sequences able to hybridise to 74 human genes/transcripts, the expression of which is altered in human calcified degenerative aortic valves [7]

GeneBank Accession /	SwissProt Accession	Gene (protein) name / function
M65199	P20800	endothelin 2
M18185	P09681	gastric inhibitory polypeptide
AB010710	P78380	oxidised low density lipoprotein (lectin-like) receptor 1
L25615	P37288	arginine vasopressin receptor 1A
Z11687	P30518	arginine vasopressin receptor 2 (nephrogenic diabetes insipidus)
D31833	P47901	arginine vasopressin receptor 1B
M31210	P21453	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1
U03865	P35368	adrenergic, alpha-1B-, receptor
L13436	P20594	natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B)
X52282	P17342	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
L02911	Q04771	activin A receptor, type I
AF015257	Q99527 Q99981 O00143 Q13631	G protein-coupled receptor 30
Y10659	P78552 Q99656 O95646	interleukin 13 receptor, alpha 1
M91211	Q15109 Q15279	advanced glycosylation end product-specific receptor
L35545	Q14218	protein C receptor, endothelial (EPCR)
AF016050	O14786 O60461	neuropilin 1
U41070	Q15722 Q13305 Q92641	leukotriene b4 receptor (chemokine receptor-like 1)
AJ002962	O15540 O14951	fatty acid binding protein 7, brain
M86917	P22059	oxysterol binding protein
S73197	P41181	aquaporin 2 (collecting duct)
L27213	P48751	solute carrier family 4, anion exchanger, member 3
U89364	P51787 Q92960	potassium voltage-gated channel, KQT-like subfamily, member 1
M20747	P14672	solute carrier family 2 (facilitated glucose transporter), member 4
U39195	P48544 Q92807	potassium inwardly-rectifying channel, subfamily J, member 5
M91368	P32418	solute carrier family 8 (sodium/calcium exchanger), member 1
M23234	P21439	ATP-binding cassette, sub-family B (MDR/TAP), member 4
J04456	P09382	lectin, galactoside-binding, soluble, 1 (galectin 1)
M93718	P29474	nitric oxide synthase 3 (endothelial cell)
X52882	P17987 Q15556	t-complex 1
X65784	Q04762	cell matrix adhesion regulator
U05291	Q06828 Q15331	fibromodulin
M58664	P25063	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
S57235	P34810	CD68 antigen
U85611	Q99828	calcium and integrin binding 1 (calmyrin)
Z34974	Q15152 O00645	plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)

Table 3 (continued)

GeneBank Accession / SwissProt Accession		Gene (protein) name / function
U49240	Q92797 O00733 O00689	symplekin; Huntingtin interacting protein I
AB000897	O15100	protocadherin gamma subfamily A, 12
AF047826	O60574	cadherin 19, type 2
U07969	Q12864	cadherin 17, LI cadherin (liver-intestine)
U59325	Q13634	cadherin 18, type 2
X52947	P17302	gap junction protein, alpha 1, 43kD (connexin 43)
M96789	P35212	gap junction protein, alpha 4, 37kD (connexin 37)
L34954	P36382	gap junction protein, alpha 5, 40kD (connexin 40)
U03493	P36383	gap junction protein, alpha 7, 45kD (connexin 45)
U34802	P48165	gap junction protein, alpha 8, 50kD (connexin 50)
X04325	P08034	gap junction protein, beta 1, 32kD (connexin 32, Charcot-Marie-Tooth neuropathy, X-linked)
M86849	P29033	gap junction protein, beta 2, 26kD (connexin 26)
X53416	P21333	filamin A, alpha (actin binding protein 280)
S73813	P49961	ectonucleoside triphosphate diphosphohydrolase 1
M90657	P30408	transmembrane 4 superfamily member 1
X82157	Q14515	SPARC-like 1 (mast9, hevin)
X87241	Q14517	FAT tumor suppressor homolog 1 (Drosophila)
Y00796	P20701	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
U81984	Q99814 Q99630	endothelial PAS domain protein 1
X07897	P02590 P04463	troponin C, slow
S64668	P45379 Q99596	troponin T2, cardiac
M14993	P11171	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)
M95627	Q13685	angio-associated, migratory cell protein
U49837	P50461	cysteine and glycine-rich protein 3 (cardiac LIM protein)
U43030	Q16619	cardiotrophin 1
M86406	P35609	actinin, alpha 2
D26512	P50281	matrix metalloproteinase 14 (membrane-inserted)
S39329	P20151	kallikrein 2, prostatic
M13143	P03952	kallikrein B, plasma (Fletcher factor) 1
L19684	P29622	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4
X14329	P15169	carboxypeptidase N, polypeptide 1, 50kD
M32313	P18405	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)
U16660	Q13011	enoyl Coenzyme A hydratase 1, peroxisomal
X07228	P78529 P11150	lipase, hepatic
U22662	Q13133	nuclear receptor subfamily 1, group H, member 3
X02750	Q16001 Q15190 Q15189 P04070	protein C (inactivator of coagulation factors Va and VIIIa)
M11723	P00748	coagulation factor XII (Hageman factor)
X68505	Q02078 Q14223 Q14224	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)

regulation of collagenases, stromelysinases, and gelatinases [67, 68] and has been found to be highly expressed in actively resorbing tissue [69]. Also, the key-role is considered for MT1-MMP as a matrix degrading protease, specifically in geometry remodelling after AVR, and opens good perspectives for new targeted therapy approaches, in order to avoid the most common metabolic impairments and clinical complications well-known to be frequently developed by the patients after AVR [53].

Acute aortic insufficiency is a frequent complication after AVR: risk assessment

Besides cases with an acute injury, e.g. aortic dissection and thoracic injury, the main aetiologies of the progressive insufficiency are bioprosthesis degeneration and infectious endocarditis [70, 71]. In order to forestall a dysfunction of degenerating bioprostheses, patients without diagnosed risk factors undergo, on average, a re-operation 9–10 years after AVR. Against this, the period of time can be more than halved for patients demonstrating at least two of following risk factors: smoking, *Diabetes mellitus*, risk by gender (females), high cholesterol level [72]. Furthermore, these risk factors have a higher impact in bioprosthesis degeneration for younger patients than for the elderly. Therefore, targeted preventive measures such as proper (pre)diabetes care would be highly beneficial, in particular for subpopulations of young female diabetes-predisposed AVR-patients.

Diabetes mellitus as the risk factor for infectious endocarditis, accelerated valvular degeneration, dysfunction of bioprostheses valves and progressive aortic insufficiency

Diabetes mellitus is a well-acknowledged risk factor for progressive aortic insufficiency, accelerated degeneration of both native and prosthetic valves as well as infectious endocarditis [72–75]. Studies focused on the aetiology and prevalence of the latter demonstrated diabetic patients to be particularly predisposed (a relative increase of 40% compared to the general population) to infectious endocarditis mainly due to following reasons:

1. patients with DM are at highly increased risk of infections
2. most patients with infectious endocarditis have a history of pre-existing heart valve lesions, which DM patients are significantly predisposed to [73, 76].

Although, both causes are considered as independent risk factors for infectious endocarditis prevalence in DM [75], the synergistic effects can lead to a “vicious circle” in further progression of infectious endocarditis, heart valve lesions/degeneration and vulnerability of DM patients for infections (see Fig. 4) [21]. Due to a high symptomatic heterogeneity of the diabetic population, the better defined “metabolic syndrome” as a cluster of atherogenic, inflammatory, and atherothrombotic abnormalities linked to abdominal obesity and insulin resistance has been demonstrated to be a

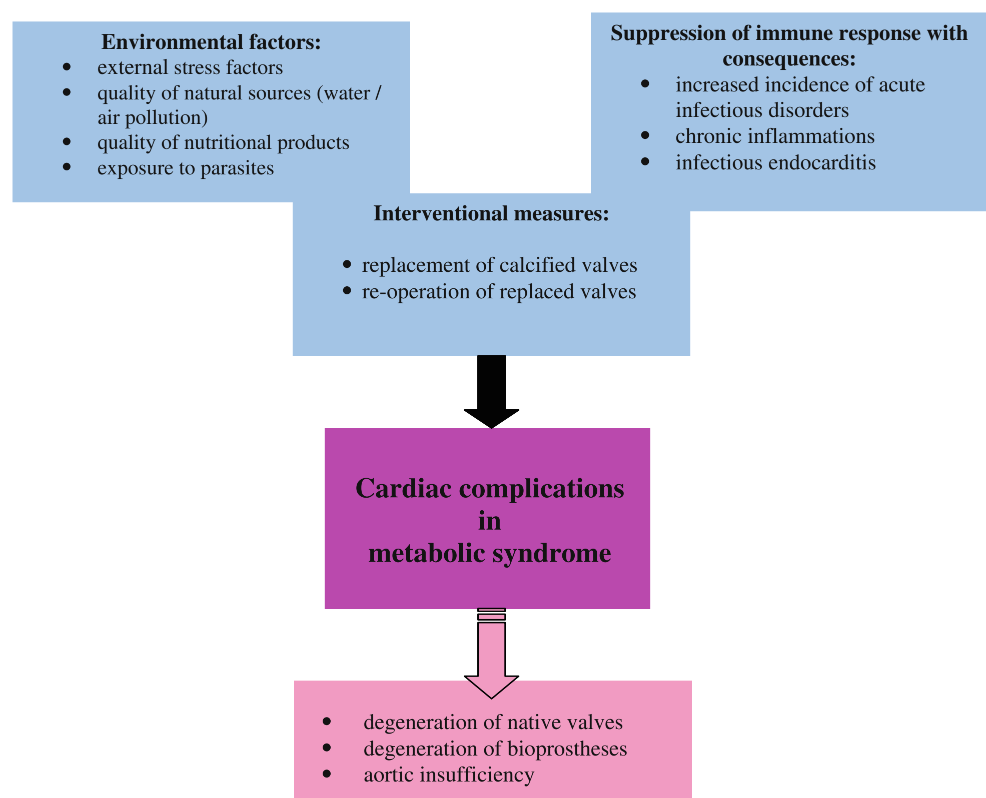

Fig. 4 Various factors, burden and pathologic processes, contributing to cardiac complications in metabolic syndrome [20]. The crucial role of environmental factors as increasing the overall risk is discussed in our previous reviews [7, 15, 21 38]

particularly strong independent predictor for poor prognosis in both degenerative valve disease and accelerated degeneration of bioprosthetic valves [73, 77]. The pro-atherogenic and pro-inflammatory pathomechanisms have been proposed to underlie the degenerative valvular processes, since statins-based treatment approaches are known to slow down the progression of valvular degeneration [73, 74, 78]. Identification of metabolic syndrome characteristic factors responsible for structural failure of a bioprosthesis is necessary for a development of individualised target-specific therapy approaches avoiding the need for re-operation after AVR. Improved (pre)Diabetes care is currently discussed as being one of the highest priorities of desirable healthcare worldwide [79–82].

Concluding remarks and Outlook

There is a long period of time during which patients predisposed to valvular degeneration remain asymptomatic. In this period a pathology progression can and must be detected followed by targeted therapeutic measures. Molecular attributes characteristic for early stages of valvular degeneration represent reliable predictive biomarkers and – at the same time – the targets for more effective individualised treatment approaches before the pathology is clinically manifested. Risk factors should be considered individually. The characteristic molecular signature is one of them.

Besides several kinds of acute injury (aortic dissection, thoracic injury) the main aetiology of the aortic insufficiency in patients after AVR is a bioprosthesis dysfunction and infectious endocarditis. On average, patients without diagnosed risk factors undergo a re-operation 9–10 years after AVR. Against this, the period of time can be more than halved for patients demonstrating at least two of following risk factors: smoking, *Diabetes mellitus*, risk by gender (females), high cholesterol levels. Therefore, individualised targeted measures would be highly effective in prevention of AVD and re-operation after AVR. Pathology- and stage-specific molecular patterns should be taken into consideration for the reliable prediction, individualised treatment algorithms and correct prognosis.

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