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Sex-dependent regulation of fibrosis and inflammation in human left ventricular remodelling under pressure overload

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Aims Women with aortic stenosis develop a more concentric form of LV hypertrophy than men. However, the molecular factors underlying sex differences in LV remodelling are incompletely understood. We took an unbiased approach to identify sex-specific patterns in gene expression and pathway regulation, and confirmed the most prominent findings in human hearts.

Methods and results Echocardiography was performed in 104 patients (53.8% women) with aortic stenosis before aortic valve replacement. LV mass, LV end-diastolic diameter, and relative wall thickness were included in a factor analysis to generate an index classifying LV remodelling as adaptive or maladaptive. Maladaptive remodelling was present in 64.6% of male and in 32.7% of female patients ($P < 0.01$). Genome-wide expression profiling of LV samples was performed in a representative subgroup of 19 patients (52.6% women) compared with samples from healthy controls ($n = 18$). Transcriptome characterization revealed that fibrosis-related genes/pathways were induced in male overloaded ventricles, while extracellular matrix-related and inflammatory genes/pathways were repressed in female overloaded ventricles (adjusted $P < 0.05$). We confirmed gene regulation by quantitative real-time reverse transcription–polymerase chain reaction and immunoblotting analysis, and we further demonstrate the relevance of our findings by histological documentation of higher fibrosis in men than in women.

Conclusion We conclude that in pressure overload distinct molecular processes are regulated between men and women. Maladaptive LV remodelling occurs more frequently in men and is associated with greater activation of profibrotic and inflammatory markers. Collectively, sex-specific regulation of these processes may contribute to sex differences in the progression to heart failure.

Keywords Gene expression • Hypertrophy • Pressure overload • Sex differences • Transcriptomics

Introduction

The response of the heart to pressure overload (PO) is hypertrophy. The initial adaptive hypertrophic response of the myocardium is characterized by structural and molecular changes, such as wall thickening, and increases in cardiomyocyte size and contractile protein content without cellular proliferation.[1] In later stages, LV hypertrophy becomes maladaptive. This maladaptive remodelling process is characterized by LV dilation and impaired function. It is a major risk factor for the development of congestive heart failure and a cause for ischaemia, arrhythmia, and sudden death.[2,3]

In PO, there are significant sex differences in LV remodelling and the transition to heart failure. Women develop more concentric hypertrophy with smaller ventricular diameter, greater relative wall

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thickness, and a better systolic function, while men demonstrate more pronounced chamber dilation.[4,5] These sex differences are partly expected to be the outcome of differences in myocardial collagen mRNA expression and architecture between males and females.[6−8] However, the molecular mechanisms underlying the sexually dimorphic response of the heart to PO, which may lead to heart failure, are incompletely understood. Moreover, inflammation is expected to contribute to sex differences in LV remodelling. In a recent study, we reported a significant increase in activation of inflammatory signalling in female mice lacking oestrogen receptor β.[9] However, little is known about the regulation of the inflammatory response in human LV remodelling.

In the present study, we hypothesized that the PO-dependent regulation of global cardiac gene expression differs markedly between men and women, and tested this in a genome-wide expression analysis. Based on resulting differences in gene regulation, we further hypothesized that the cardiac response to PO in men is mainly characterized by increases in fibrosis and inflammation, while we expected less pronounced effects in women. We anticipate that unravelling the sex differences in molecular processes underlying PO will be useful for the development of strategies for the prevention of heart failure after surgery, including transcatheter aortic valve replacement (TAVR), where women constitute 50% of the patients,[10] and may contribute towards the design of more appropriate and personalized therapies for men and women.

Methods

Study population

A cohort of 104 patients (53.8% women) with isolated aortic stenosis and preserved EF undergoing conventional aortic valve replacement were included in the present study. Patients with a history of structural or infectious myocardial disease or other co-morbidities, such as malignancies or systemic diseases that may affect LV remodelling, were excluded. The study was approved by the Charite University Hospital Ethics Committee, complies with the principles outlined in the Declaration of Helsinki, and written consent was obtained from all patients.

Echocardiography

The echocardiographic measurements were taken 1 week before aortic valve replacement according to the guidelines of the American Society of Echocardiography (ASE) and the European Society of Echocardiography (ESC). LV mass and LVEF were calculated using standard procedures,[11,12] i.e. according to the Devereux formula, and relative wall thickness was calculated using the formula 2 × posterior wall thickness/LV diastolic diameter. We estimated mean and maximum aortic valve pressure gradients by the modified Bernoulli equation, by application of the flow velocity time integral over the ejection period in continuous wave Doppler recordings with a 100 mm/s time scale on the *x*-axis for all patients.

Hybridization and microarray profiling

Biopsies from the lateral LV wall of 19 aortic stenosis patients (52.6% women) were collected during aortic valve replacement and frozen immediately in liquid nitrogen until further processing. Samples from the lateral LV wall of non-diseased human hearts technically unusable for transplantation obtained from general organ donors were frozen immediately in liquid nitrogen until further processing and used as controls [$n = 18$; 44.4% female; mean age = 55.6 years (men) and 55.8 years (women)] for comparisons with male and female diseased LV biopsies, respectively, as performed previously.[13−15] To avoid potential sources of error and the introduction of bias and variation into the study due to technical reasons, we were consistent with tissue sampling, i.e. location, collection, processing, and storage, in all groups. Experimental protocols were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB: 4991-0/2010-1018EKU).

Total RNA was isolated using the RNeasy Fibrous Tissue Mini kit (Qiagen) following the manufacturer's protocol. RNA quality and quantity were established using a 2100 Bioanalyzer (Agilent Technologies) ensuring adequate RNA quality and that all samples, i.e. healthy and diseased, can be compared. Biotinylated complementary RNA (cRNA) was prepared and hybridized to the Human Gene 1.0 ST array (Affymetrix) according to the standard Affymetrix processing protocol. The array was scanned in a GeneChip Scanner 3000. The quality of hybridization was assessed in all samples following the manufacturer's recommendations. Microarray data are deposited in the ArrayExpress database under accession number E-MEXP-3684.

Microarray data analysis

The computational and statistical analysis of the microarray data was carried out using the R version 2.14.2 software[16] and the Bioconductor packages,[17] as described recently.[18,19] Following background correction, expression data were normalized with the variance stabilization and normalization algorithm,[20] and $\log_2$ transformed using the median polish algorithm of robust multiarray average.[21] The quality of the data was assessed with the *affy*[22] and the *arrayQualityMetrics*[23] packages. We ensured adequate quality of the entire resulting data set using standard quality metrics and graphical tools, confirming that there were no outliers and that diseased and control tissues could be compared. To detect differences in transcript cluster expression between conditions, a moderated linear model was applied using the *limma* package.[24] To adjust resulting *P*-values, the false discovery rate (FDR) was controlled. Pathway analysis was performed by means of gene set enrichment analysis[25,26] querying the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[27] and by using the *Category* and *GSEABase* packages. The distribution was computed based on 1000 permutations adjusting for multiple testing. Statistical significance was considered at FDR-adjusted $P \leq 0.05$ at all times.

Quantitative real-time RT–PCR

Reverse transcription and quantitative real-time PCR were performed as described previously.[28,29] Total RNA was reverse transcribed and amplified using the One Step RT qPCR MasterMix (Eurogentec) in a LS480 Lightcycler (Roche). Reactions where RNA or reverse transcriptase had been omitted were used as negative controls. PCR products were obtained using gene-specific, intron-spanning primers. The levels of all candidate genes were normalized to ribosomal protein L32 (*RPL32*) housekeeping mRNA levels. Primer sequences used for amplification and product sizes are shown in Supplementary material online *Table S6*.

Histology

The LV tissue samples were fixed in 4% formaldehyde, embedded in paraffin, cut into 3 μm sections and stained with chromotrope aniline blue (CAB) using standard procedures.

Immunoblotting

The LV samples were homogenized in a modified RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Na-deoxycholate) supplemented with protease inhibitor cocktail (Roche), and the phosphatase inhibitors sodium orthovanadate (1 mM Na_3VO_4) and sodium fluoride (1 mM NaF). Proteins were quantified using the BCA Assay (Pierce), resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transferred to nitrocellulose membranes, and incubated with antibodies against CD3 (Thermo Scientific) and tubulin (Sigma). Immunoreactive proteins were detected using ECL Plus (GE Healthcare).

Statistical analysis

All data were analysed statistically using the SPSS version 21 and R version 2.14.2 software. Multivariate normality of the data was tested using the E-statistic.[30] Comparisons between two groups were performed with unpaired *t*-test or Mann–Whitney U-test, and between multiple groups using two-way analysis of variance with Tukey's post-hoc test adjusting for multiple comparisons. Categorical variables were tested using χ^2 test. A *P*-value ≤ 0.05 was considered statistically significant.

Factor analysis was used to analyse LV remodelling under conditions of chronic PO.[31,32] Following ASE guidelines, LV end-diastolic diameter (LVEDD) was used as a marker for dilation, and LV mass and relative wall thickness (RWT) as markers for concentricity. Mean and peak transvalvular pressure gradients were also included. The measures related to LV remodelling were combined in the LV remodelling index and forced orthogonal to the PO index (combines mean and peak transvalvular pressure gradients). The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was 0.374, while the Bartlett's test of sphericity was highly significant ($P < 0.0001$). Principal component analysis with Varimax rotation was used as the underlying factor-analytical method. The Kaiser–Guttman criterion was applied to ascertain the number of extracted factors. We used the LV remodelling index to classify echocardiographic LV remodelling into adaptive or maladaptive patterns. Since the LV remodelling index is a *z*-transformed variable, positive index values correlate with an increase in LVEDD (factor loading 0.962) and LV mass (factor loading 0.661) and a decrease in RWT (factor loading −0.818), thereby reflecting maladaptive LV remodelling. The LV remodelling pattern was considered adaptive when the criteria for maladaptive LV remodelling were not fulfilled, i.e. negative LV remodelling index values.

Results

Patient characteristics and sex-specific left ventricular remodelling

The study population was composed of 48 men and 56 women (*Table 1*). The prevalence of diabetes mellitus, hyperlipidaemia, hypertension, and CAD was similar between men and women. Women compared with men had significantly smaller LV end-diastolic diameter and LV mass, and greater RWT. LVEF and transvalvular pressure gradients were similar between the sexes.

Our analysis of LV remodelling patterns revealed a statistically significant difference between men and women. In particular, maladaptive remodelling was present in 64.6% of men, while it was present in 32.7% of women (*Figure 1*).

Table 1 Baseline characteristics of the study population

	Total ($n = 104$)	Men ($n = 48$)	Women ($n = 56$)	*P*-value
Age, years	70 ± 9.4	69 ± 10	70 ± 9	0.836
Body mass index, kg/m^2	28 ± 4.5	28 ± 3.6	28 ± 5.2	0.758
Systolic blood pressure, mmHg	136 ± 18	137 ± 19	136 ± 17	0.657
Diastolic blood pressure, mmHg	72 ± 11	74 ± 8	70 ± 13	0.052
Diabetes mellitus, %	29	33	25	0.350
Hyperlipidaemia, %	57	52	61	0.376
Hypertension, %	88	88	88	1.000
Coronary artery disease, %	5	8	2	0.179
LVEDD, mm	48 ± 6	50 ± 5	46 ± 6	0.001
Left ventricular mass, g	233 ± 59	250 ± 56	217 ± 58	0.004
Relative wall thickness	0.5 ± 0.08	0.48 ± 0.07	0.52 ± 0.09	0.036
Mean pressure gradient, mmHg	58 ± 12	57 ± 11	58 ± 13	0.654
Maximal pressure gradient, mmHg	83 ± 19	85 ± 16	81 ± 21	0.290
Left ventricular ejection fraction, %	62 ± 4	61 ± 4	62 ± 5	0.189
ACE inhibitors, %	45	45	45	0.997
Beta-blockers, %	50	47	54	0.494
Diuretics, %	40	43	38	0.602

Values are shown as mean ± SD or %.
LVEDD, left ventricular end-diastolic diameter

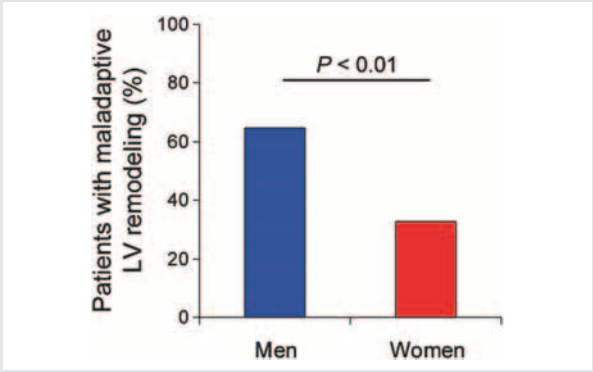

Figure 1 Proportion of male and female patients with maladaptive LV remodelling. The LV remodelling index (see the Methods) revealed that a significantly greater number of male patients ($n = 48$) had maladaptive remodelling than female patients ($n = 56$). $^{**}P < 0.01$.

Transcriptome analysis reveals sex-specific regulation of fibrotic and inflammatory genes/pathways in human left ventricular remodelling

Following genome-wide expression profiling of intraoperative myocardial biopsies in a representative subgroup of 19 patients (52.6% women; maladaptive LV remodelling: 60% men, 40% women), we obtained valid data on 32 321 transcript clusters. Applying an FDR-adjusted *P*-value threshold of 0.05, we identified 2873 transcript clusters regulated in male tissues, while 3491 transcript clusters were regulated in female tissues (*Figure 2A*). Of these, 1382 transcript clusters were significantly regulated in both sexes, while the remaining clusters were specific for each sex (*Figure 2A*). This demonstrates that although there is a substantial overlap in gene regulation, there are significant differences in the genomic response to PO between men and women.

Within the genes regulated in both male and female tissues, several known markers associated with PO and LV hypertrophy can be found (Supplementary material online, *Table S1*), including the myosin light chain 4 (*MYL4*), natriuretic peptide A (*NPPA*), and myocyte enhancer factor 2A (*MEF2A*) genes. However, examining the behaviour of the commonly regulated genes, we discovered that the extent of the regulation of a number of these genes differed considerably between the sexes (*Figure 2B* and *C*). Considering genes whose behaviour was most different between men and women, we found that among others, extracellular membrane (ECM)- and fibrosis-related genes were induced to a higher degree in male PO tissues than in female PO tissues compared with controls (*Figure 2D*), while inflammation-related genes were repressed to a higher degree in female PO tissues than in male PO tissues compared with controls (*Figure 2E*).

Functional enrichment analysis using Gene Ontology terms revealed that in male tissues the expression of genes belonging to fibrotic processes, such as collagen fibril organization, transforming growth factor-beta (TGF-β) receptor, and the bone morphogenic protein (BMP) signalling pathway, was increased. On the other hand, in female tissues, the expression of inflammatory genes involved in regulation of B cell-mediated immunity and regulation of alpha–beta T-cell activation was repressed.

Employing the KEGG database, we found that the ECM–receptor interaction, peroxisome proliferator-activated receptor (PPAR), p53, TGF-β, and FcεRI signalling pathways were induced in male PO tissues only (adjusted $P < 0.05$; *Table 2* and Supplementary material online, *Table S4*). On the other hand, we found that a large number of inflammatory pathways, such as the cytokine–cytokine receptor interaction, cell adhesion molecules, natural killer cell-mediated cytotoxicity, phagosome, Toll-like receptor, and Jak–STAT signalling pathways, were repressed in female PO hearts (adjusted $P < 0.05$; *Table 2* and Supplementary material online, *Table S5*).

Confirmation of increased fibrosis and inflammation in male hearts

Due to the prominent sex differences in ECM/fibrosis-related and inflammatory genes/pathways identified by our whole-transcriptome analysis, we selected candidates within these biological processes for the validation of the resulting high-throughput data. Quantitative real-time RT–PCR confirmed that although the expression of the ECM gene asporin (*ASPN*)[33] was significantly increased in both male and female PO tissues compared with controls, this increase was significantly higher in male than in female tissues (*Figure 3A*). Furthermore, as in the genome-wide expression profiling, the expression of TIMP metallopeptidase inhibitor 2 (*TIMP2*), chemokine (C-X3-C motif) receptor 1 (*CX3CR1*), interleukin 33 (*IL33*), and Toll-like receptor 7 (*TLR7*) was increased in male tissues only, and the expression of TGF-β1 (*TGFB1*) was decreased in female tissues only (*Figure 3A*). In line with these gene expression patterns, male tissues displayed much more increased fibrous, pericellular collagen depositions than female tissues as illustrated by the CAB staining (*Figure 3B*). Moreover, immunoblotting analysis demonstrated that the protein levels of proinflammatory CD3 were higher in male than in female tissues (*Figure 3C*). Taken together, these data demonstrate the validity of our findings by confirming the selected candidates from the whole-transcriptome analysis through different experimental approaches.

Discussion

Differences in the development, progression, and outcome of PO-induced LV hypertrophy between men and women have been well documented and have long been expected to arise from different molecular mechanisms active between the sexes. Our work is the first to show how the molecular response to PO differs between men and women and that the male heart increases fibrosis and inflammatory pathways, while the female heart suppresses fibrosis-related and inflammatory processes.

Initially, we performed differential expression analysis at the single gene level comparing the LV transcriptome of male and female

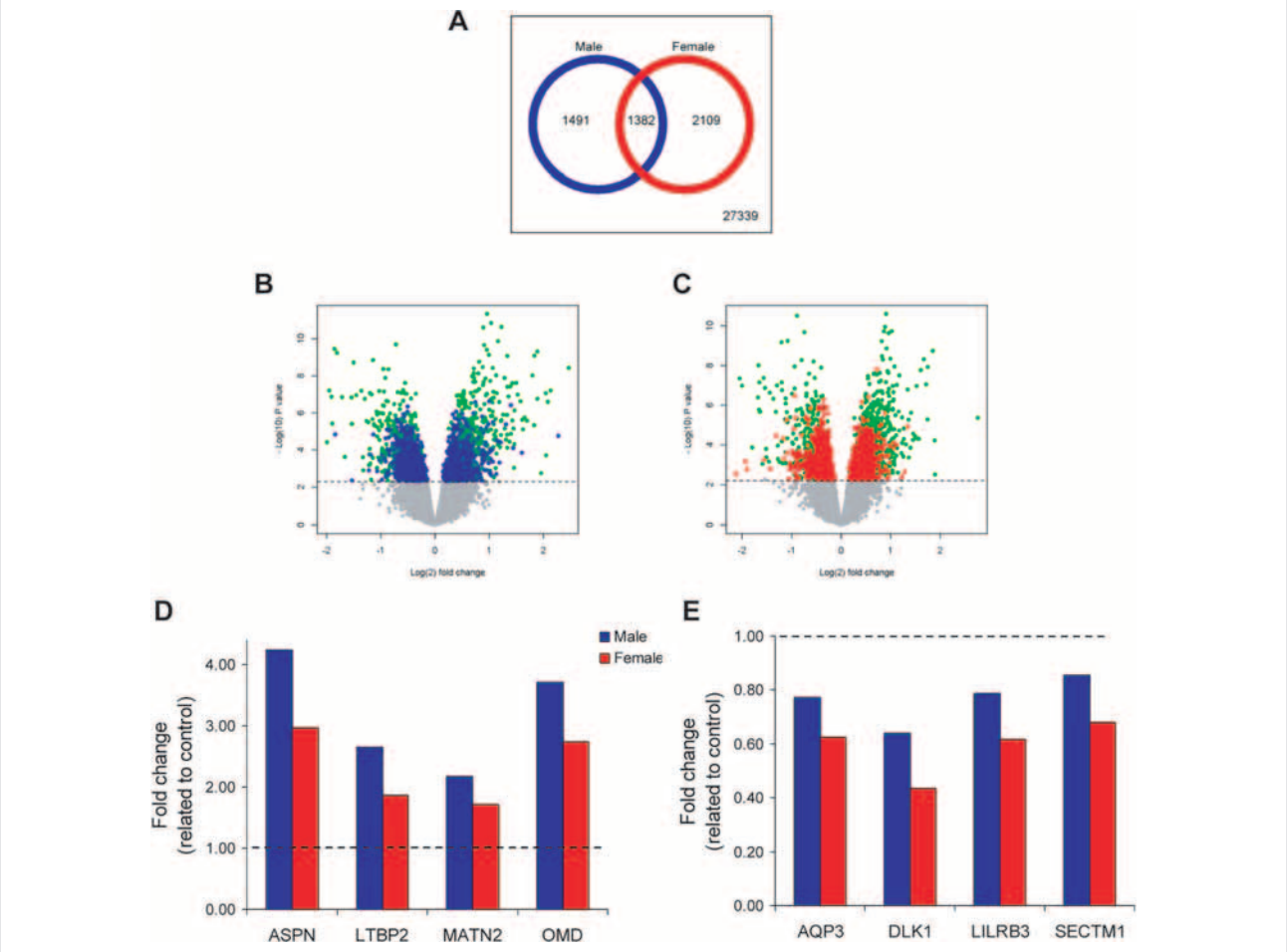

Figure 2 Comparison of differential gene expression. (*A*) Venn diagram showing the number of transcript clusters regulated in cardiac tissues of male and of female patients and those regulated in common compared with controls. (*B*) and (*C*) Volcano plots of significance of genes against their relative change in expression. Circles represent all transcript clusters present in the data set. For each transcript cluster, the negative $\log_{10}$ of the *P*-value is plotted against the relative change in gene expression. The dashed horizontal line indicates the significance threshold ($P < 0.05$) after false discovery rate correction for multiple testing. Transcripts regulated in male tissues only are shown in blue (*B*), those regulated in female tissues only are in red (*C*), and those that were regulated in common are shown in green for both sexes. (*D*) and (*E*) Expression plots for extracellular matrix (ECM)-, fibrosis-, and inflammation-related genes. In pressure overload, ECM- and fibrosis-related genes were induced more in male tissues than in female tissues (*D*), while inflammation-related genes were more repressed in female tissues than in male tissues (*E*) compared with controls. Data are shown as fold change related to control. $n = 8–10$/group. *ASPN*, asporin; *LTBP2*, latent transforming growth factor beta binding protein 2; *MATN2*, matrilin 2; *OMD*, osteomodulin; *AQP3*, aquaporin 3; *DLK1*, delta-like 1 homologue; *LILRB3*, leucocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3; *SECTM1*, secreted and transmembrane 1.

patients with the LV transcriptome of men and women with no cardiovascular disease. As anticipated, several genes were regulated in both male and female tissues. For instance, the expression of *NPPA* and *MEF2A* was increased in response to PO. This was expected, as both genes have a prominent role in LV remodelling, demonstrating the validity of our approach.[34,35] However, we found a number of genes commonly regulated in male and female tissues that had a different extent of regulation between the sexes. This was the case with ECM- and fibrosis-related genes, which had higher expression in male than in female aortic stenosis tissues. The stronger induction of these genes in male tissues supports the view that a higher degree of fibrosis occurring in the hearts of male patients with aortic stenosis may play a key role in the worse outcome observed in men.

Fibrogenesis is a necessary process that follows stress and injury, such as myocardial infarction and hypertrophy. Consequently, initial activation of fibrotic factors is crucial for the heart to maintain its integrity and function. However, persistent fibrosis and excessive ECM remodelling contribute to disease progression and may lead to heart failure. The hallmark of fibrosis is the differentiation of fibroblasts to myofibroblasts, which in turn mediate tissue remodelling. Myofibroblasts can be activated by TGF-β, angiotensin II, and other cytokines. To this extent, we found that the ECM–receptor

Table 2 Top 10 Kyoto Encyclopedia of Genes and Genomes pathways regulated in male or female diseased vs. control left ventricular tissues

ID	Name	Adjusted *P*-value	Regulation
Male			
04512	Extracellular matrix–receptor interaction	<0.001	Up
04614	Renin–angiotensin system	<0.001	Up
04622	RIG-I-like receptor signalling pathway	<0.001	Up
04350	Transforming growth factor-beta signalling pathway	0.005	Up
04510	Focal adhesion	0.005	Up
04310	Wnt signalling pathway	0.006	Up
03010	Ribosome	<0.001	Down
04260	Cardiac muscle contraction	0.002	Down
03050	Proteasome	0.004	Down
00190	Oxidative phosphorylation	0.005	Down
Female			
03430	Mismatch repair	0.006	Up
04146	Peroxisome	0.011	Up
04060	Cytokine–cytokine receptor interaction	<0.001	Down
04062	Chemokine signalling pathway	<0.001	Down
04514	Cell adhesion molecules (CAMs)	<0.001	Down
04610	Complement and coagulation cascades	<0.001	Down
04620	Toll-like receptor signalling pathway	<0.001	Down
04630	Jak–STAT signalling pathway	< 0.001	Down
04650	Natural killer cell-mediated cytotoxicity	< 0.001	Down
04145	Phagosome	0.001	Down

interaction, renin–angiotensin system, and TGF-β signalling pathways were activated only in male patients. Furthermore, our analysis at the single gene level demonstrated that the gene with the highest fold increase in male PO tissues compared with controls was periostin (*POSTN*). Periostin is an ECM protein activated when fibroblasts convert to myofibroblasts and is a mediator of fibrosis.[36,37] Cardiac-specific overexpression of periostin increases LV collagen deposition, while its deletion decreases LV hypertrophy and fibrosis.[37] In addition, the expression of many more ECM proteins, such as collagen type I and III, was increased in the hearts of men with aortic stenosis. The broad activation of profibrotic molecular mediators therefore denotes that excessive fibrosis and ECM remodelling occur in male hearts, contributing to severe LV dilation and cardiac dysfunction.

In contrast, female hypertrophied hearts showed an active suppression of profibrotic factors. In particular, the gene encoding TGF-β itself (*TGFB1*) was repressed, as well as that of angiotensinogen (*AGT*), the precursor of angiotensin II. Moreover, we observed a co-ordinated and generalized repression of chemokine- and cytokine-related and inflammatory pathways. These could be key mechanisms against the progression of fibrosis, as it has been shown that fibroblast progenitor cells, known as fibrocytes, are recruited to the heart and mediate fibrosis.[38] Cardiomyocytes may produce cytokines, promoting a profibrotic environment. Furthermore, chemokines and their receptors are crucial for fibrocyte and leucocyte migration. Therefore, in addition to the evident protection against the detrimental effects of increased inflammation through the inhibition of proinflammatory factors, the present findings suggest that female PO-stressed hearts may be further protected against uncontrolled fibrosis, preventing fibrocyte migration through the reduced expression of cell adhesive molecules. The overall validation of ECM/fibrosis-related and inflammatory markers through different experimental approaches underscores the robustness of these findings.

Limitations

Our transcriptome analysis was constrained by a relatively small sample size stratified by sex, related to limited heart material available from closely matched living individuals. However, the internal consistency and validation of our findings by different experimental approaches support the conclusions demonstrating differences in the cardiac gene signature between men and women in PO. A further limitation is the deviation between the KMO measure of sampling adequacy and the Bartlett's test of sphericity. While the KMO measure was not satisfactory, the Bartlett's test indicated strong relationships among the assessed variables appropriate for factor analysis.

Conclusions

In conclusion, the present study demonstrates how the response of the cardiac transcriptome to PO differs between men and women. In view of the pronounced sex-specific regulation of fibrotic

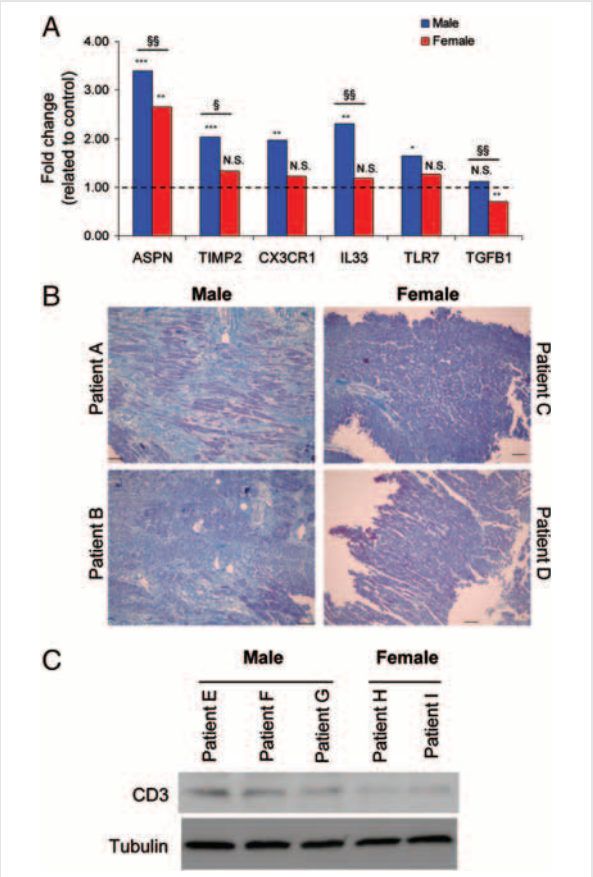

Figure 3 Validation of genome-wide expression profiling. (*A*) Relative mRNA levels of selected candidates were quantified by qRT–PCR in LV samples from male and female patients and normalized to ribosomal protein L32 (*RPL32*). Since there were no sex differences at basal expression, data are shown as fold change related to control. $n = 9–10$/group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for diseased vs. control; $^{\S}P < 0.05$ and $^{\S\S}P < 0.01$ for male diseased vs. female diseased. *ASPN*, asporin; *TIMP2*, TIMP metallopeptidase inhibitor 2; *CX3CR1*, chemokine (C-X3-C motif) receptor 1; *IL33*, interleukin 33; *TLR7*, Toll-like receptor 7; *TGFB1*, transforming growth factor-beta 1. (*B*) Representative chromotrope aniline blue (CAB)-stained LV sections showing collagen deposition (light blue) in two male and two female patients. Scale bar = 100 μm. (*C*) Representative western blotting analysis of LV homogenates from three male and two female patients. Levels of CD3 protein were determined using a specific antibody. Tubulin served as loading control.

and inflammatory markers, we anticipate that these are major underlying mechanisms responsible for the differences observed in the clinical outcome of PO-induced stress between men and women, thereby influencing the progression to heart failure. We put forward that sex-specific therapeutic interventions may be of increased value towards a more personalized medical care. Furthermore, the integration of biomarkers for both fibrosis and inflammation might be a clinical tool useful for early identification of changes in these pathways.

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Supplementary Information

Additional Supporting Information may be found in the online version of this article:

TableS1. Transcript clusters regulated in both male and female PO tissues compared with controls.

TableS2. Transcript clusters regulated in male PO tissues compared with controls.

TableS3. Transcript clusters regulated in female PO tissues compared with controls.

TableS4. Pathways regulated in male PO tissues compared with controls.

TableS5. Pathways regulated in female PO tissues compared with controls.

TableS6. Primer and probe sequences.

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Conflict of interest: S.G. and H.S. are employees of Bayer HealthCare. All other authors have no conflicts to declare.

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