Differential gene expression in patients with primary mitral valve disease: identifying potential therapeutic targets in the era of precision medicine

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This data were previously presented as an oral presentation at the American Association for Thoracic Surgery Mitral Conclave.

Accepted 18 August 2020
Published Online First 6 September 2020

To cite: Shih E, Squiers JJ, Turner J, *et al*. *J Investig Med* 2020;**68**:1289–1291.

ABSTRACT

Primary (degenerative) mitral valve (MV) disease is a result of structural remodeling due to degenerative and adaptive changes of MV tissue. We hypothesized that in patients with primary MV disease undergoing surgery for severe mitral regurgitation (MR), a distinct genetic expression profile within the MV leaflet tissue could be identified as compared with patients without MV disease. Tissue samples from the MV leaflets of 65 patients undergoing MV surgery for MR due to primary MV disease and 4 control cadavers without MV disease were collected and analyzed. MicroRNA transcripts were hybridized to Illumina HumanHT-12 v4 Beadchips. Ingenuity pathway analyses (IPAs) were conducted to provide biological interpretation. Of the approximately 20 000 genes examined, 4092 (20%) were differentially expressed between patients with primary MV disease and normal controls (false discovery rate<0.05). The differentially expressed genes could be clustered into five regulator effect networks from the Ingenuity Knowledge IPA database with a consistency score of >6. These five networks have been previously implicated in pathophysiological cardiac abnormalities, including inhibited contractility of the heart and fatty acid oxidation as well as activation of apoptosis of smooth muscle cells, cardiac degeneration, and hypertrophy of cardiac cells. MV tissue in patients with primary MV disease demonstrated distinct genetic expression patterns as compared with normal controls. Further studies are necessary to determine whether the molecular pathways identified in this experiment may represent potential therapeutic targets to prevent degeneration of MV tissue leading to severe MR.

INTRODUCTION

Mitral valve (MV) prolapse is the leading cause of isolated mitral regurgitation (MR).[1] MV degeneration leading to prolapse may be sporadic or familial, with some data favoring an autosomal dominant inheritance pattern.[2] Fibromyxomatous changes in mitral leaflet tissue lead to increases in area and length of the tissue, eventually causing MV leaflets to bulge superiorly into the left atrium with respect to the annular ring in the absence of acquired leaflet disease. Geometrical changes secondary to MV degeneration eventually cause prolapse and regurgitation, ultimately progressing to heart failure.

MV prolapse in patients with heritable disorders such as Ehlers-Danlos, Marfan, and Loeys-Dietz syndromes is attributed to mutations in various structural proteins and their regulatory genes. However, little is understood about the molecular processes involved in the pathophysiology of MV prolapse outside the scope of these specific disease processes. We sought to use microarray analysis of native MV tissue to identify genetic expression pathways unique to patients with MV prolapse compared with patients with no known valvular disease.

METHODS

We obtained MV tissue from 66 patients undergoing MV repair or replacement for primary MV disease at Baylor Scott & White The Heart Hospital according to an institutional review board-approved protocol (#010–162). Adult patients with confirmed MV prolapse by echocardiography within the prior 6 months requiring surgical intervention were eligible. Exclusion criteria included patients with conditions known to predispose the patient to MR, including coronary artery disease or fibroelastic disorders (eg, Marfan syndrome). The control group consisted of MV tissue samples from four cadavers with no known valvular disease.

At the time of the surgery, tissue samples were resected from MV leaflets. Tissue samples were stored in RNAlater solution (Ambion, Austin, Texas, USA) at 4°C for 24 hours and then transitioned to −80°C until processing for RNA isolation. Microarray analysis was performed using Illumina HumanHT-12 v4 Beadchip system to identify differences in individual genetic expression between the two cohorts. A false discovery rate (FDR) of ≤0.05 was set as the parameter for differential expression. Other statistical analyses, including Bonferoni, raw

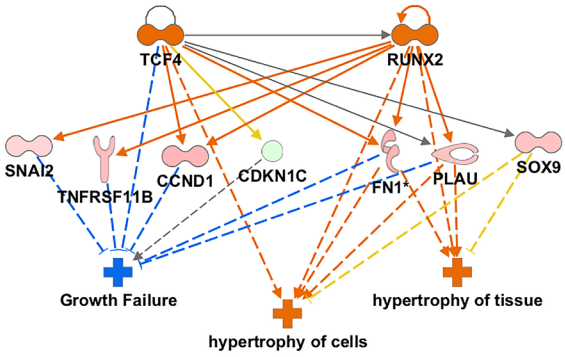

Figure 1 Genetic regulatory pathways that may contribute to MV disease progression.

p values, and FDR≤0.10, were also performed to ensure the integrity of the primary analysis. Identified genes were summarized into their regulatory pathways by ingenuity pathway analysis (IPA). This method uses 'big data' analysis to connect identified expression patterns with known regulatory networks linked to disease/pathology from the currently published molecular biology literature (figure 1). A calculated consistency score (Z-score) makes predictions about regulatory genes based on up/down gene-regulation patterns and their activation/inhibition patterns.[3]

RESULTS

Patients had a mean age 64±14 years, and 77% were male. Patients in the control group (cadavers with no known valvular disease) had a mean age 51±5 years at their time of death, and 50% were male. A total of 4092 genes were differentially expressed with FDR≤0.05. More conservative statistical tests yielded fewer differentially expressed genes, which was consistent with expected outcomes (table 1). These genes were evaluated with IPA to identify five regulator effect networks with high consistency scores (table 2).

DISCUSSION

We performed microarray analysis to identify differential genetic expression in MV tissue between patients with primary MV prolapse and controls without valvular disease. Five regulatory networks were identified that may be associated with MVP. It is unclear whether the variations in genetic expression within MV tissue identified in our study are the cause or the result of MVP (or unrelated). However, this study adds several potential therapeutic targets to the list of previously suggested pathways involved in the development of MV disease.[4 5] This preliminary analysis provides a baseline for future confirmatory validation studies of targeted genetic pathways.

Table 1 Differential gene expression by microarray analysis

Statistical analysis	Genetic targets
Raw p value≤0.5	6195
FDR≤0.5	4092
FDR≤0.10	5161
Bonferoni≤0.5	652

FDR, false discovery rate

Table 2 Ingenuity pathway analysis of regulator effect networks

ID regulators	Diseases and functions	Consistency score
Foxp1, GATA5, HAND2, HEY2, MYOCD, TBX5, THRA	Contractility of heart ventricle	8.758
ESRRA, KLF15, NR1D2, SRA1, TFAM	Degeneration of heart and glucose metabolism disorder	7.845
GATA4, HAND2, MEF2A, MEF2C, MYOCD, TBX5, THRA	Apoptosis of smooth muscle cells	7.6
RUNX2, TCF4	Growth failure and hypertrophy of cells	6.425
FOXO3, MEF2C, RNX2, SMAD7, TCF4	Hypertrophy of cardiac muscle	6.102

Higher consistency score of predicted regulatory network shows more consistency with current literature.[3]

Our study is subject to several limitations. First, the sample size of the control group (n=4) may limit the reliability of the results, although how much is difficult to quantify beyond the statistical analysis already presented. It is important to highlight, however, several important factors related to the size of the control group. Acquiring MV tissue from patient cadavers with known non-diseased hearts is challenging because these hearts are often transplanted, and thus studies of this nature often have a limited number of controls available. In contrast, our disease cohort (n=66) is substantially larger than was available in previously published studies using similar genetic analyses to investigate MV pathology.[4] Finally, there was a 7-year age difference between the diseased cohort and healthy controls for which no adjustment was performed in our analyses. We suspect that this difference in age is unlikely to factor largely into differences in genetic expression related to a disease that takes decades to develop in vivo (and at least one previous study found no correlation between age and genetic expression in MV samples[4]), but further investigation is necessary to confirm this hypothesis.

Our study is subject to several limitations. The study is limited by the small sample size of our control group. There is also a notable difference in mean age between the two groups in the study. The effect of age on genetic expression in MV prolapse is uncertain and warrants further study.

Delineation of the pathophysiology of MV prolapse requires further investigation. Our goals were to identify potential genetic regulatory pathways that may contribute to MV disease progression and to guide future studies that may identify molecular pathways that can be therapeutically targeted to slow or prevent MV degeneration.

Contributors All listed authors made significant contributions to the manuscript that meet the four criteria widely used by the International Committee of Medical Journal Editors to determine authorship. Specifically, ES and JJS helped to design the study, collect and analyze data, and drafted and revised the manuscript. JT performed key data analysis and interpretation, as well as revised the manuscript. MD, WTB, and RLS each assisted with data collection and analysis, study design, and revisions of the manuscript. All

authors provided final approval and agreed to be accountable for all aspects of the work.

Funding This research was supported in part by a grant from the Cardiovascular Research Review Committee of the Baylor Foundation.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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