SECOND LINE PHENOTYPING TO CHARACTERIZE A SPONTANEOUS DEGENERATIVE MITRAL VALVE DISEASE IN FVB MICE

Authors : **Estelle Ayme-Dietrich[1], Sylvia Da Silva[1], Ghina Alame Bouabout[2], Alizée Arnoux[1], Jérôme Guyonnet[3], Guillaume Becker [1], Laurent Monassier [1]**

1 Laboratoire de Pharmacologie et Toxicologie Neuro Cardiovasculaire UR7296, Fédération de Médecine Translationnelle, Centre Hospitalier Universitaire et Université de Strasbourg, Centre de Recherche en Biomédecine de Strasbourg (CRBS), Strasbourg, France,
2 Mouse Clinical Institute. PHENOMIN, CNRS UMR7104, INSERM U964, Université de Strasbourg. 1 rue Laurent Fries, BP 10142 Parc d'innovation 67404 ILLKIRCH Cedex France
3 Pharma Innovation Department, CEVA Santé Animale, Libourne Cedex, France

Background:

Mitral valve disease is a complex entity with diverse origins. The degeneration can occur following an extrinsic aggression (infection, autoimmune attack, drug), malformation or due to a hemodynamic abnormality (dilatation of the left ventricle, myocardial infarction).

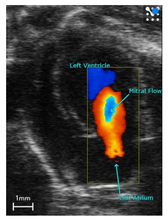
In this work, we characterized the spontaneous mitral valve degeneration of FVB/NJ mouse sub-strain in comparison with the C57BL/6J, the usual background strain of many cardiovascular studies with cardiac phenotyping.

Conclusions:

The FVB mouse strain is highly prone to spontaneous mitral myxomatous degeneration. A contribution of the peripheral serotonergic system is suggested.

Materials and Methods:

Males FVB/NJ were compared to putative C57BL/6 control at 24-week-old. Body weight, systolic blood pressure, heart rate, urinary 5-hydroxyindoleacetic acid (5-HIAA), whole blood and plasma serotonin, tail bleeding time, blood cell count, plasma TGF-β1 and plasma natriuretic peptide concentrations were measured. Myocardium and mitral valves were characterized by histology. The Cardiac anatomy and function were assessed by high frequency echocardiography. To know if the mitral valve was remodeled, myocardium and mitral valves were characterized by histology.



Results:

Compared to C57BL/6J, FVB mice strains present:

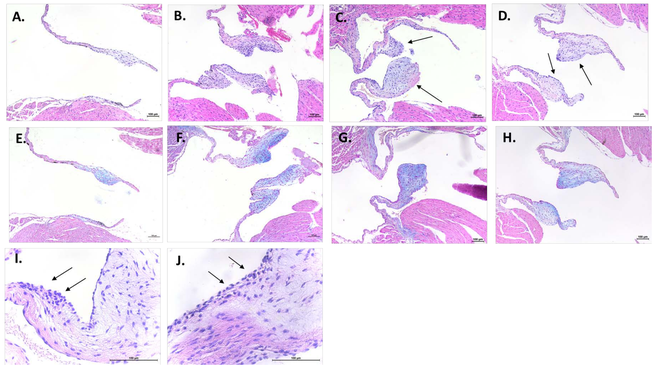
- No significantly differ regarding body weight increase, arterial blood pressure and heart rate.
- A progressive augmentation of plasma pro-ANP.
- No cardiac hypertrophy or left-ventricular fibrosis were observed.
- High prevalence of fibromyxoid highly cellularized and enriched in glycosaminoglycans lesions, inducing major mitral leaflets thickening without increase in length.
- Increased thickness was correlated with urinary 5-HIAA
- Whole blood serotonin concentration was similar in the two strains
- A reduction of plasma serotonin in FVB was observed together with an increase of the bleeding time.
- Left atrial and left ventricular remodeling identified by echocardiography associated with thickening of both mitral leaflets and mitral insufficient in 30% of FVB mice but no systolic protrusion of mitral leaflets towards the atrium.

Physical, hemodynamical and biochemical parameters obtained in males

Wk 24		
	C57BL/6J	FVB/NJ
BW	30.8±0.9	31.4±1.0
SBP	107±4	110±5
HR	715±12	701±11
Pro-ANP	0.4±0.0	1.1±0.4
TGF	19±11	32±18
HIAA	32±4	48±10

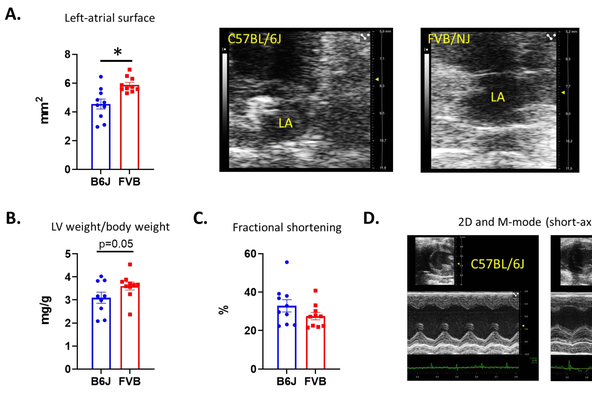
BW: body weight (g), SBP: systolic blood pressure (mmHg), HR: heart rate (bpm), Pro-ANP: plasma pro natriuretic peptide type A (nmol/L), TGF: plasma transforming growth factor-β1 (x103 pg/dL), HIAA: urinary 5-hydroxyindole acetic acid (µmol/L)

Spontaneous mitral valve lesions in C57BL/6J and FVB mice

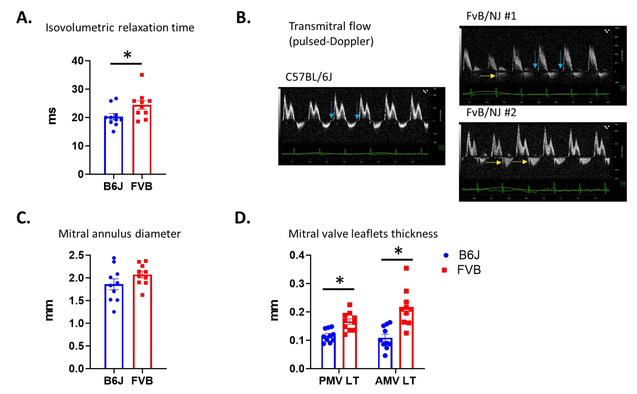


Hematoxylin and eosin staining shows thickened leaflets, called "cushions", with important cellular density, in FVB/NJ (C) and FVB/NRj (D) mice, in comparison to thin leaflets in C57BL/6J mice (A). However, some C57BL/6J mice may have highly cellularized valvular lesions (B), but with a prevalence of only 30% and a lower thickness. In C57BL/6J (E and F), as well as in FVB mice (G and H), Alcian blue staining shows severe deposition of glycosaminoglycans (blue) in the spongiosa and disrupted, disorganized collagen (pink); all features that are characteristics of fibromyxoid lesions. Finally, clusters of rounded shape cells bordering the valvular surface and penetrating the interstitial matrix were observed in all mice with lesion, but more prominent in FVB mice (I and J) (scale bar=100µm).

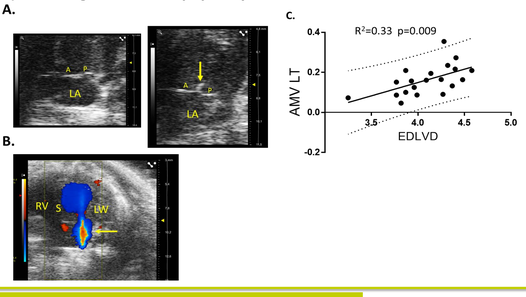
Echocardiographic evaluation showing left cardiac cavities, systolic, diastolic left ventricular function and anatomo-pathologic parameters of mitral apparatus



In FVB/NJ mice compared to C57BL/6J echocardiography identified the enlargement of the left atrium (A). Echocardiographic pictures are showing C57BL/6J (upper and left panel) and FVN/NJ (upper and right panel) left atrium obtained in a 2D apical view. An important dilation of the left-atrium is observed in the FVB/NJ mouse. We also observed a left-ventricular hypertrophy (B). At that stage, no reduction of the systolic function was observed (C). In D, the increased thickness of the ventricular walls can be observed on the 2D (upper picture) and the M-mode obtained in a mid-ventricular short axis section of the left ventricle in a C57BL/6J (left) and a FVB/NJ (right). A disorganization of papillary muscles can also be observed in the FVB/NJ mouse case.



A trouble of the cardiomyocytes primary relaxation as attested by the isovolumetric relaxation time increase (A) was observed in FVB/NJ animals. This increase is shown in the FVB/NJ case #1 (blue arrows) (B). In the two FVB/NJ mice a mitral insufficiency is detected with a pulsed-Doppler window placed at the tip of the leaflets meaning that the insufficiency is originating at the valve's commissure (yellow arrows). Mitral valve annulus is not enlarged (C) but leaflets are thickened (D).



The absence of mitral valve prolapse is demonstrated by the lack of systolic valvular protusion towards the atrium in FVB/NJ (right) compared to C57BL/6J (left) (apical view). Note the mitral lesions observed at the tip of mitral leaflets (arrow) in the FVB/NJ case (A). Some FVB mice show massive mitral valve insufficiency as shown by the systolic backflow from the ventricle to the atrium observed with color Doppler (yellow arrow). The red part in the middle of the blue backflow corresponds to aliasing due to high flow velocities (apical view) (B). The remodeling of the mitral valve is associated with the dilatation of the left ventricle (C). A: anterior leaflet of the mitral valve, P: posterior leaflet of the mitral valve, LA: left atrium, S: interventricular septum, RV: right ventricle, LW: lateral wall of the left ventricle, AMVLT: Anterior Mitral Valve Leaflet Thickness, EDLVD: End-Diastolic Left-Ventricular Diameter.