Tetralogy of Fallot: Physiological and morphological changes in conditional Jagged1 mutant mice

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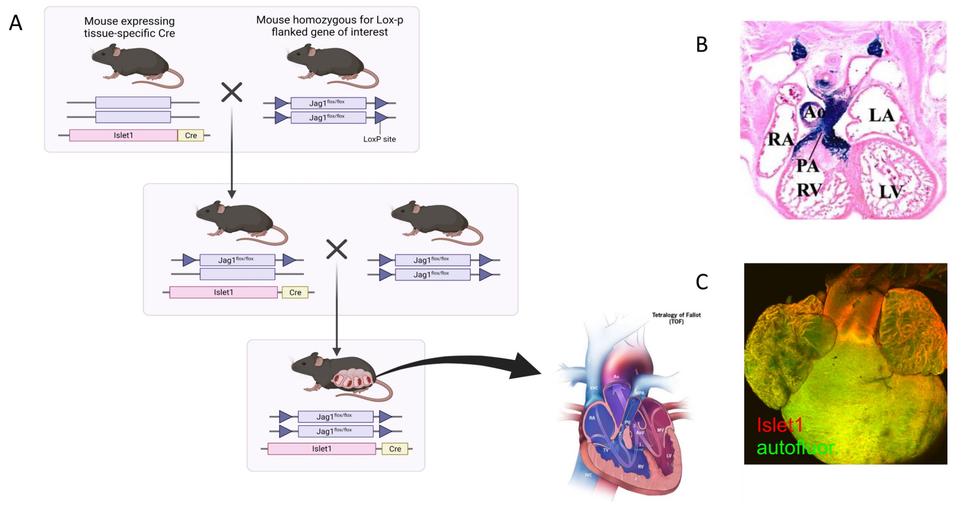
Introduction

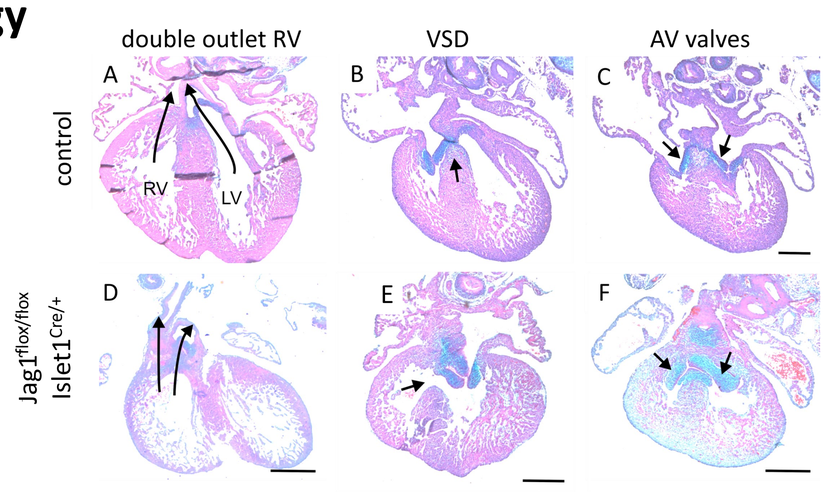
Congenital heart diseases, the majority of which affect the right-sided or pulmonary circulation, contribute significantly to mortality in patients with Alagille syndrome. Mutations in the human Jagged1 (Jag1) gene, which encodes a ligand for the Notch receptor, have been observed in the majority of patients . Symptoms of this inherited disease may include various forms of Tetralogy of Fallot. To better understand the role of Jag1 in normal heart development we generated mice model simulating the Tetralogy of Fallot.

Model



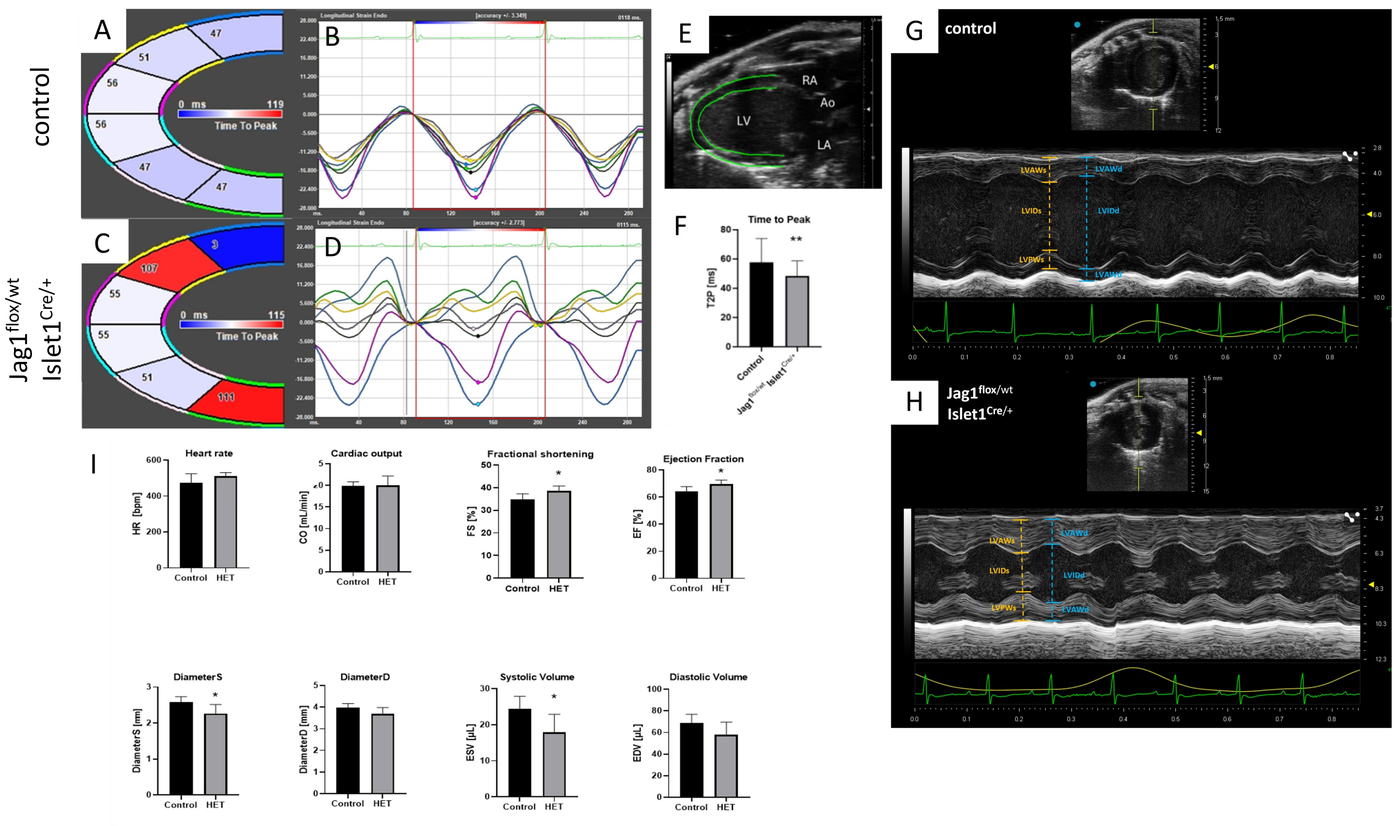
We generated Jag1$^{flox/flox}$ Islet1$^{Cre/+}$ mice (A) with targeted Jag1 gene conditional deletion in heart progenitor cells of the secondary heart field and neural crest cells, which mainly participate in the formation of right ventricle and outflow tract (OFT), together with pulmonary and aortic valves (B, blue, Sun et al., 2007). Islet1 is also expressed in the wall of main stems of coronary arteries as well as in cardiac nerves. The expression of Islet1 in our model was visualized using RosaTdTomato Islet1$^{Cre/+}$ mouse (C, red) .

Morphology



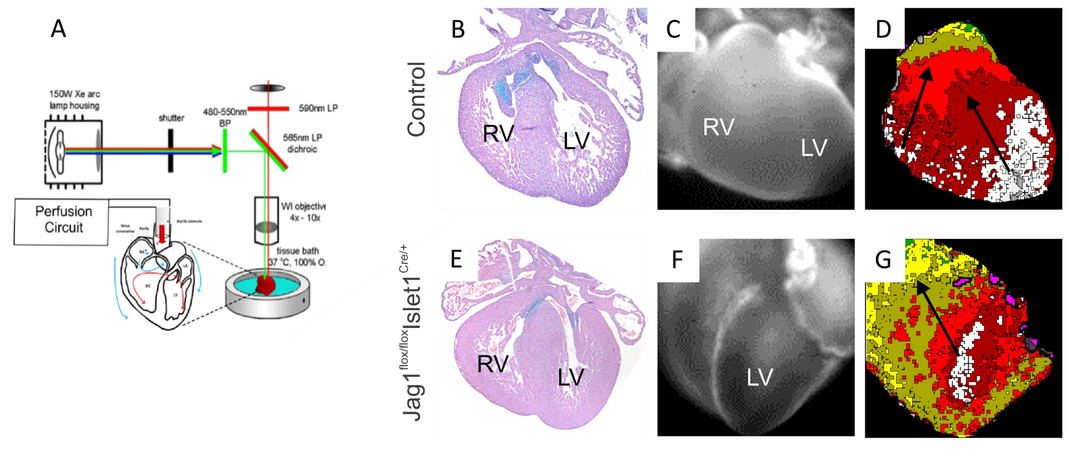
Normal development of ED14.5 embryo with no double outlet (A), with fully closed interventricular septum (IVS, B) and with normal atrio-ventricular (AV) valves (C). Jag1$^{flox/flox}$ Islet1$^{Cre/+}$ with double outlet right ventricle (D), presence of membranous VSD (E) and thickening and malformations of AV valves (F). Scale bars 200 µm.

VEVO ultrasound imaging

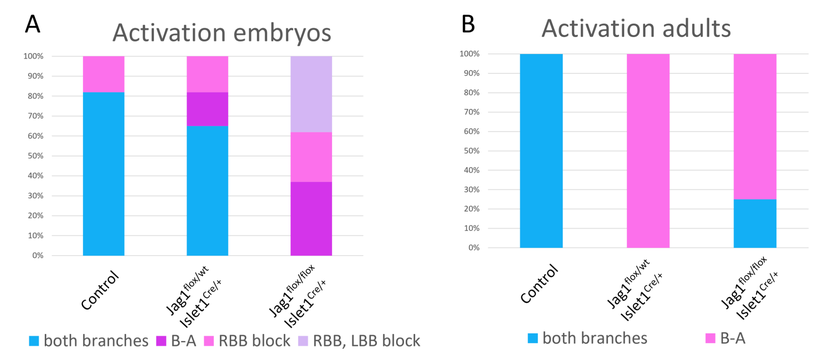


Left ventricle (LV) strain analysis was conducted using B-Mode images acquired from the parasternal long axis view (E). Speckle tracking was utilized to trace the endocardial and epicardial borders for each LV segment (A,C). The colors of the segment edges (A, C) correspond to the colors of the lines in panels B and D, representing the temporal progression through the cardiac cycle. The analysis results of a control mouse (A, B) and Jag1$^{flox/wt}$ Islet1$^{Cre/+}$ (C, D). In Jag1$^{flox/wt}$ Islet1$^{Cre/+}$(E, F), dysynchrony indicated by varying T2P compared to controls is evident, it also manifested as asymmetry among curves (D). Graph F quantifies strain as the time from baseline to peak strain. Short axis view and M-mode tracing of the LV (G, H), revied LV hypertrophy in Jag1$^{flox/wt}$ Islet1$^{Cre/+}$. Echocardiography show trend in increase of the heart rate and cardiac output (I). Fractional shortening and ejection fraction is significantly increased in Jag1$^{flox/wt}$ Islet1$^{Cre/+}$. Systolic diameter is significantly decreased, however diastolic diameter is not significantly decreased in Jag1$^{flox/wt}$ Islet1$^{Cre/+}$. Systolic volume is significantly decreased in Jag1$^{flox/wt}$ Islet1$^{Cre/+}$. LVAW = Left ventricle anterior wall, LVID = Left ventricle internal diameter, LVPW = Left ventricle posterior wall, -d = in diastole. -s = in systole.

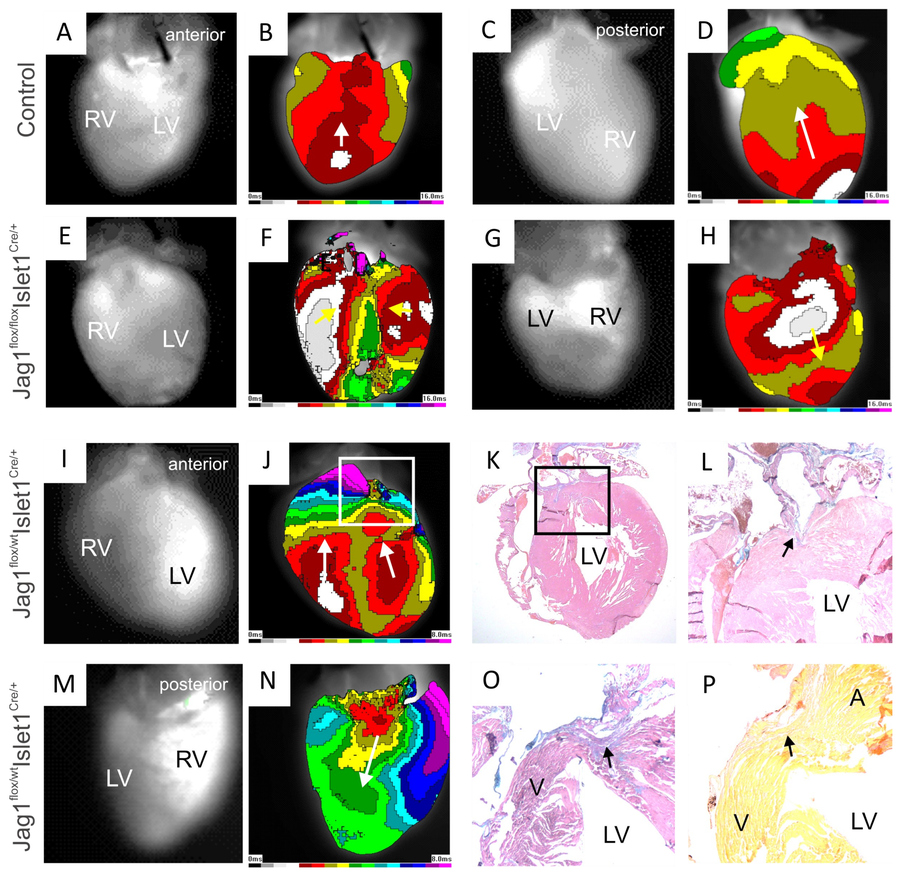
Optical mapping



Set-up for optical mapping of retrogradely perfused heart (A). Control heart (B) with normal activation pattern from both bundle branches (RBB and LBB) (C, D). Jag1$^{flox/flox}$ Islet1$^{Cre/+}$ heart (E) with activation from LBB (RBB block) (F, G).



Activation patterns of embryonic hearts (A), activation patterns with normal activation via both branches, abnormal activation from base to apex (B-A) but with activation via both branches and activation only with right or left bundle branch (RBB or LBB). Activation patterns in adult hearts (B) – normal activation via both branches from apex to base and abnormal activation from base to apex with disrupted activation of posterior heart aspect (B-A).



The adult control heart is activated from the apex and the signal propagate to the base (A, B, C, D). Jag1$^{flox/wt}$ Islet1$^{Cre/+}$ is activated by LBB and RBB (E, F) and posteriorly from middle of the ventricle (G, H). Irregularities in Jag1$^{flox/wt}$ Islet1$^{Cre/+}$ activation of OFT (I, J), continuity of myocardium in OFT visualized by histology ABHE (K, L). Posteriorly Jag1$^{flox/wt}$ Islet1$^{Cre/+}$ hearts are activated from base to apex (M, N), lack of fibrous insulation between atrium and ventricle myocardial continuity (arrows), histology ABHE (O), picrosirius red staining (P).

Conclusion

Jagged1 conditional knockout in secondary heart field results in:

- Double outlet right ventricle
- Ventricular septal defect
- Valve abnormalities
- Improper electrical insulation of atrio-ventricular junction, which is manifested as persisting myocardial connections
- lower T2P of longitudinal strain in the epicardium
- Ventricular dysynchrony
- Cardiac hypertrofy

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