Congenital Heart Disease: The Leading Cause of Infant Mortality

Cardiac Abnormalities in Mouse Litters With Double Mutation of Inversin & Vangl-2 Genes

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Introduction

- Heart alignment and septation defects constitute the largest group of cardiac malformations and both can be caused by Inversin (INV) / Vangl-2 genes disruption.[1]
- Vangl-2 gene is a core factor in the planar cell polarity mechanism, which regulates the cell migration during the heart development. It mainly responsible for a spectrum of cardiac outflow tract defects in the event of gene mutation. [1]

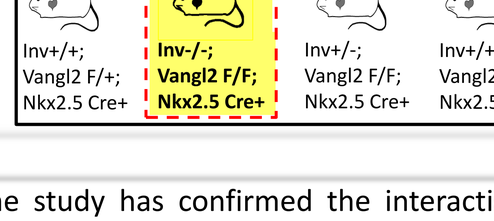


Figure 2: Cardiovascular defects of the Vangl2 mutant looptail. In looptail homozygotes **(B)** the great arteries are parallel rather than spiraling as normal in heterozygote littermates **(A)**. Aorta **(ao)**, pulmonary truck **(pt)**.

- Since the heart formation is exquisitely sensitive to the disturbance of the left-right symmetry, any disruptions to the INV gene will result in mirror image orientation of the normal heart and abdominal organs (Situs inversus totalis).[2]

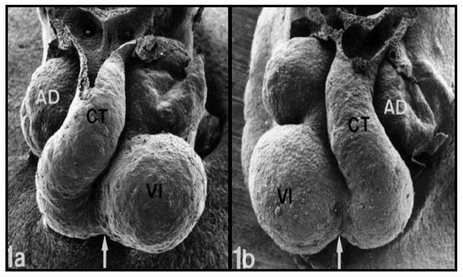


Figure 1: Scanning electron microscopy. Mouse hearts, E10.5. 1*a)* situs solitus (normal heart orientation); 1*b)* situs inversus totalis. right atrium**(AD)**; left ventricle**(VI)** and cono-trunk**(CT)** .

Aim

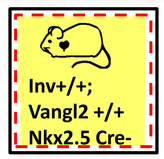
To identify the interaction effects between Inv and Vangl-2 genes in the heart development.

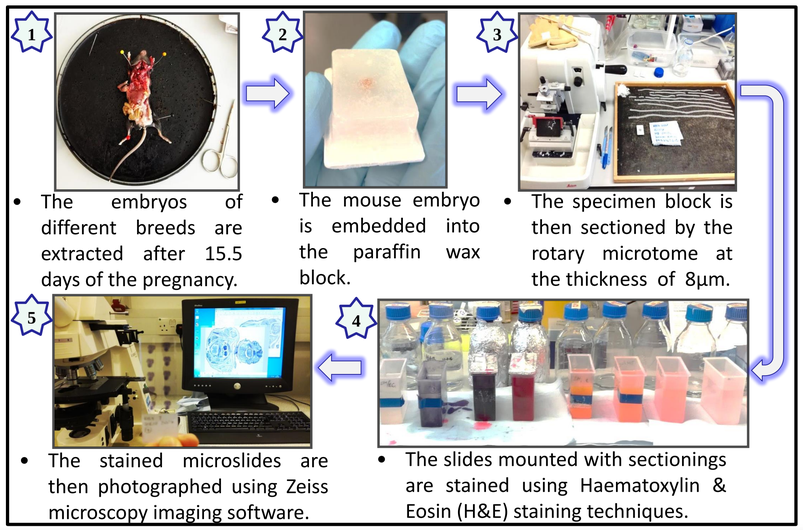
Methods

- The first mouse of interest with the genotype of **Inv-/-; Vangl2 +/+; Nkx2.5 Cre+** is bred by knocking out INV gene without disrupting the Vangl2 gene throughout all the body cells.

- The second mouse of interest with the genotype of **Inv -/- ; Vangl2 F/F; Nkx2.5 Cre+** is bred by cross breeding parents with the genotypes as shown in the figure.

- The third breed line of mouse with the genotype of **Inv+/+; Vangl2 +/+; Nkx2.5 Cre-** has acted as the control in this experiment.





- The embryos of different breeds are extracted after 15.5 days of the pregnancy.
- The mouse embryo is embedded into the paraffin wax block.
- The specimen block is then sectioned by the rotary microtome at the thickness of 8µm.
- The slides mounted with sectionings are stained using Haematoxylin & Eosin (H&E) staining techniques.
- The stained microslides are then photographed using Zeiss microscopy imaging software.

Conclusion

The study has confirmed the interaction between INV & Vangl2 genes in the cardiovascular development. The heart has expressed combined genes' defects in the event of double gene mutations.

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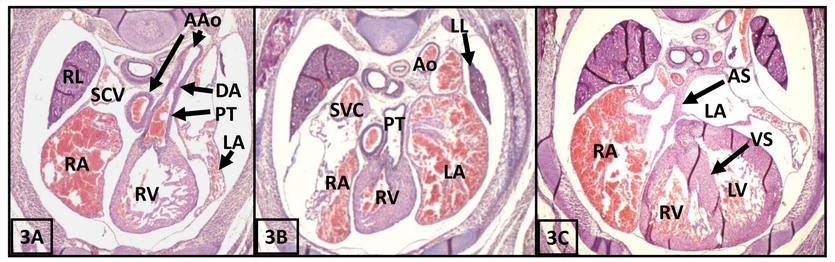
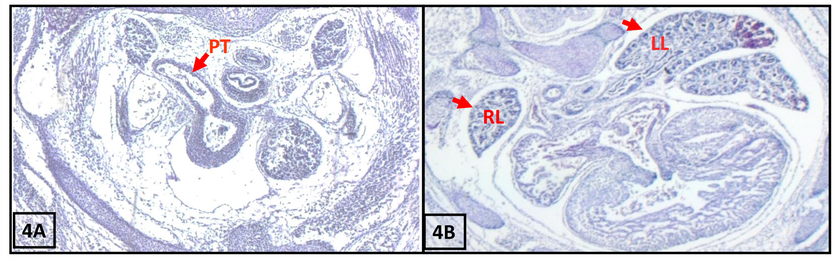
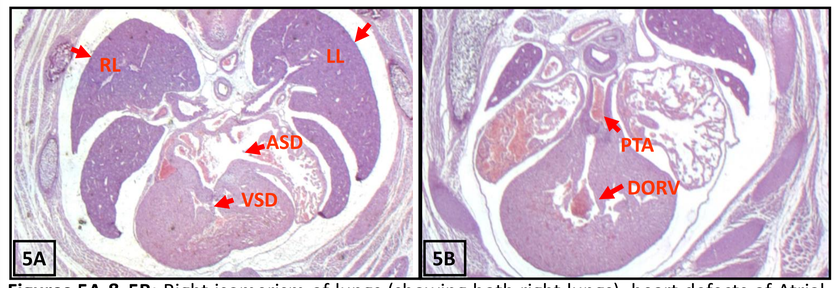
Results



Figures 3A,3B & 3C: The normal and control mouse **(Inv+/+; Vangl2 +/+ Nkx2.5 Cre-)** transverse heart level sectioning. Right Atrium**(RA)**; Right Ventricle**(RV)**; Left Atrium**(LA)**; Pulmonary Trunk**(PT)** at left; Ductus Arteriosus**(DA)**; Arch of Aorta**(AAo)**; Superior Vena Cava**(SVC)**; Right Lung**(RL)**; Left Lung**(LL)**; Atrial Septum**(AS)**; Ventricular Septum**(VS)**



Figures 4A & 4B: Heart abnormalities which the pulmonary trunk at right observed in **(4A)** and mirror-image reversal of lungs in **(4B)** are indications of situs inversus totalis. These abnormalities are seen in all the 5 subjects **(Inv-/-; Vangl2 +/+ Nkx2.5 Cre+).**



Figures 5A & 5B: Right isomerism of lungs (showing both right lungs), heart defects of Atrial Septal Defect**(ASD)** and Ventricular Septal Defect**(VSD)** are seen in **(5A)**. Persistent Truncus Arteriosus**(PTA)** and Double Outlets Right Ventricle**(DORV)** are shown in **(5B)**. These abnormalities are not observed in all the 5 subjects **(Inv-/-; Vangl2 F/F Nkx2.5 Cre+).**

	Mouse litter numbers									
	Inv-/-; Vangl2 +/+; Nkx2.5 Cre+					Inv-/-; Vangl2 F/F; Nkx2.5 Cre+				
Defects	28	33	49	8.8	13.7	1.5	3.5	8.2	11.6	13.4
ASD							✓	✓		
VSD						✓	✓	✓		
Right Isomerism							✓	✓		✓
DORV						✓	✓	✓		
PTA							✓	✓	✓	✓
Mirror Image of Lungs	✓	✓	✓	✓	✓					
Right Pulmonary Trunk	✓	✓	✓	✓	✓					

Table 1 shows the summary of heart and lungs abnormalities observed in all the mouse subjects in the project.

References
1.Ramsbottom SA, Sharma V, Rhee HJ, Eley L, Phillips HM, Rigby HF, et al. Vangl2-regulated polarisation of second heart field-derived cells is required for outflow tract lengthening during cardiac development. PLoS Genet [Internet].2014 2014/12//; 10(12):[e1004871 p.].
2. Morgan D, Goodship J, Essner JJ, Vogan KJ, Turnpenny L, Yost JH, et al. The left-right determinant inversin has highly conserved ankyrin repeat and IQ domains and interacts with calmodulin. Human Genetics. 2002;110(4):377-84.