Interaction of Inversin and Vangl2 to cause laterality and congenital heart defects

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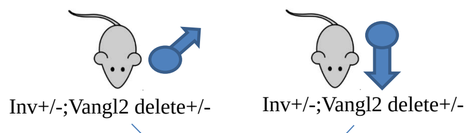
Introduction

- Congenital Heart Defects are the most common cause of infant mortality due to a birth defect.
- Disruption of inversin (Inv) is known to be responsible for either partial or complete disturbance of laterality. Disturbances are rarely seen except in homozygote recessive Inv mice. [2-3]
- Vangl2 is a gene responsible for cell polarity and double recessive mutants are often seen to have severe outflow tract defects. [1]
- Heterozygote offspring for both genes individually shows no phenotype. An initial study was carried out to find whether double heterozygous mice would have a cardiac phenotype and thus suggest a possible interaction between these two genes.

Aim: Investigate the possible interaction between Inversin and Vangl2 in double heterozygous mice for these genes

Methods

- Mouse Cross



Expected Genotypes	Number
Inv+/+; Vangl2 delete +/+	3
Inv+/-; Vangl2 delete +/+	2
Inv+/-; Vangl2 delete +/-	10
Inv+/+; Vangl2 delete +/-	3
Inv+/+; Vangl2 delete -/-	8
Inv-/- ; Vangl2 delete +/-	1
Inv-/-; Vangl2 delete -/-	1

Table 1: Depicting possible genotypes that could be obtained and number of genotypes observed. Highlighted in yellow are double heterozygotes

- Procedure

E15.5 Embryos were collected → Embryos were embedded in wax → Microtome was used to section wax blocks → Sections were stained using Hematoxylin & Eosin → Stained sections were viewed and imaged using a bright field microscope

Observed phenotypes for double heterozygotes Inv+/-;Vangl2 delete+/-

- 10 double heterozygous mice (Inv +/-;Vangl2 +/- delete as seen in Table 2) were obtained during the course of the project and were analysed. Laterality and cardiac phenotypes were determined and are shown in the table below.
- Inv+/+;Vangl2 delete +/+ mice were used as controls as depicted in Table 1.

Embryo number	Stomach Position	Ventricle Apex	VSD	AVSD
1.3	R	L	Yes	Yes
1.4	L	L	Yes	No
1.5	L	L	Yes	Yes
2.3	L	L	No	No
2.5	R	L	Yes	No
2.9	L	L	No	No
3.1	L	R	Yes	No
3.3	L	L	Yes	No
4.2	L	L	Yes	No
4.3	R	L	No	No

Table 2: Depicting Inv+/- Vangl2 +/- embryos.
Stomach position and apex of ventricle are used to determine laterality. 4 mutants show laterality disturbances.
Cardiac phenotypes were observed with 7 having ventricular septal defects (VSD) and 2 having atrioventricular septal defects (AVSD). These are an indication of cardiac defects.. In two cases both AVSD and VSD were observed.

Conclusion

- This study demonstrates that there is a possible interaction between Inversin and Vangl2 since heterozygotes on their own show no phenotype but when together a cardiac phenotype is observed.

Discussion and Future Work

- A point of interest during the project was analysing the Inv-/- Vangl2 -/- mutant. Only one embryo was obtained. This may be due to a potentially lethal effect of the double mutants early in development and as a result they do not survive to e15.5. The embryo that survived did not show a cardiac phenotype. Re genotyping of the double mutant embryo would ensure that the correct genotype is obtained.
- Further, more embryos need to be collected to see if any more double mutants can be obtained for analysis.
- To confirm whether there is an actual interaction between the genes, we can use immunohistochemistry to observe if the proteins colocalise within the heart. If we see that the proteins are present at the same time point during development. Further, coimunoprecipiataion can be used to see if the proteins physically interact.

VSDs were observed in double heterozygotes

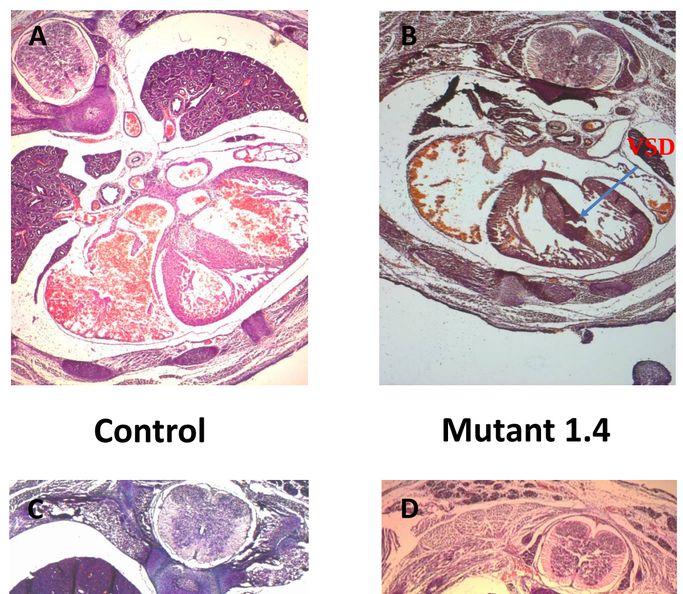


Figure 1: Examples of Ventricular Septal Defects seen in 15.5 mutants

A, C and E represent the anatomy of the heart in three different wild type embryos acting as controls.
B, D and F represent the anatomy of three different mutants with Ventricular Septal Defects.
F shows Perimembranous VSD. This means that the septum does not completely separate the two ventricles.

Laterality defect were observed in double heterozygotes

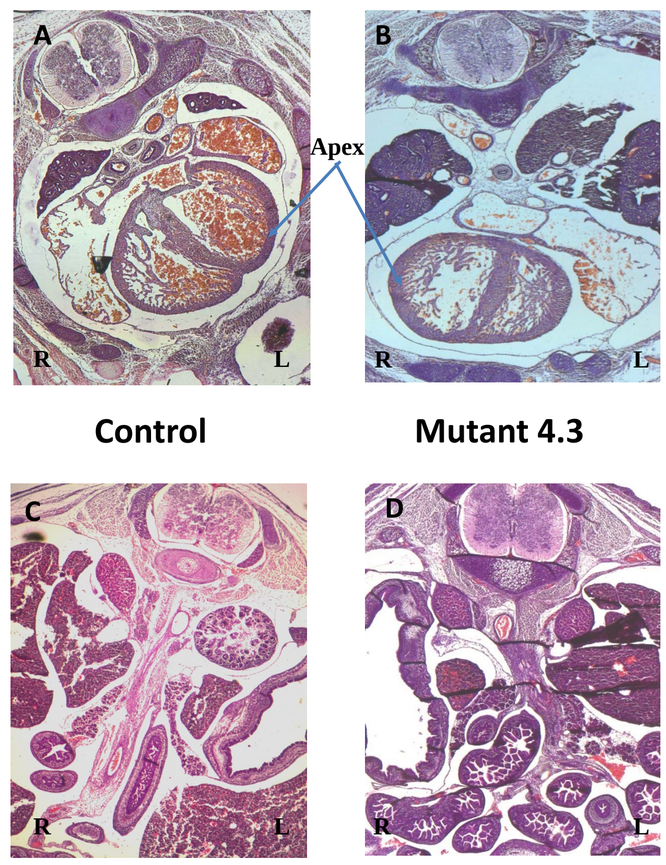


Figure 2: Examples of laterality defects seen in 15.5 mutants.
A and C represent the heart and stomach of controls respectively. As expected, both the apex of ventricle and stomach appear towards the left. B and D represent the heart and stomach of mutant embryos. It is visible in B that the apex of the ventricle is pointed towards the right, and the stomach is localised towards the right in D. R=Right, L=Left

AVSDs were observed in double heterozygotes

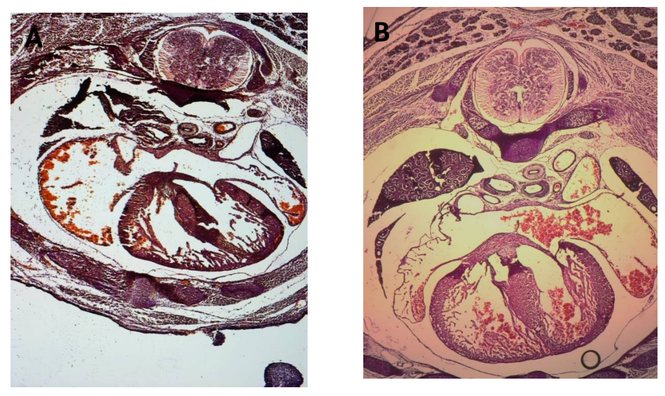


Figure 3: Examples of Atrioventricular Septal defects in 15.5 mutants

References

1. Ramsbottom, S. A., Sharma, V., Rhee, H. J., Eley, L., Phillips, H. M., Rigby, H. F., … Henderson, D. J. (2014). Vangl2-Regulated Polarisation of Second Heart Field-Derived Cells Is Required for Outflow Tract Lengthening during Cardiac Development. PLoS Genetics, 10(12), e1004871.
2. Mochizuki, T., Saijoh, Y., Tsuchiya, K., Shirayoshi, Y., Takai, S., Taya, C., Yonekawa, H., Yamada, K., Nihei, H., Nakatsuji, N., et al., 1998. Cloning of inv, a gene that controls left/right asymmetry and kidney development. Nature 395, 177–181.
3. Morgan D, GoodshipJ, EssnerJJ, VoganKJ, TurnpennyL, Yost JH, et al. The left-right determinant inversinhas highly conserved ankyrinrepeat and IQ domains and interacts with calmodulin. Human Genetics. 2002;110(4):377-84.

Acknowledgements

I would like to thank Mashael Alrardi and Dr. Lorraine Eley for support in the lab. Further, I'd like to thank Professor Deborah Henderson for the opportunity and conceptualisation of the project. Lastly, I'd like to thank Newcastle University for awarding me the Vacation Scholarship and funding the project.