Bicuspid aortic valve and hypoplastic aorta as a result of *Pax9* gene deletion in mice.

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Introduction

DiGeorge Syndrome is caused by a deletion on chromosome 22 and patients suffer from a wide range of disabilities, including those affecting the heart and its great vessels. One of the deleted genes, *TBX1*, affects the expression of another gene, *Pax9*, and we hypothesise that *Pax9* may be a genetic modifier of the DiGeorge Syndrome cardiovascular phenotype. To investigate the role of *Pax9* in development we have been studying a transgenic mouse model lacking this gene. In April 2012 a paper was published describing an infant male patient with a hemizygous *Pax9* deletion, who had a complex congenital heart malformation, including a hypoplastic aorta and bicuspid aortic valve (Santen et al, 2012). To determine whether the cardiovascular phenotype presented by this patient was also present in a transgenic mouse model of *Pax9* deletion, I have studied *Pax9*-null embryos using histological techniques, comparing them to control embryos (heterozygous for the *Pax9* mutation; $Pax9^{+/-}$).

Aims

1) Determine a method to measure the aorta of control ($Pax9^{+/-}$) and mutant ($Pax9^{-/-}$) embryos from histological sections, and determine whether there are any significant differences between the controls and the mutants.
2) Investigate whether the loss of *Pax9* in mice affects the development of the aortic valve, causing it to be bicuspid or tricuspid.
3) Examine the origin of the coronary arteries to see whether they are normal in the *Pax9*-null embryos.

Cardiovascular defects in *Pax9*-null embryos

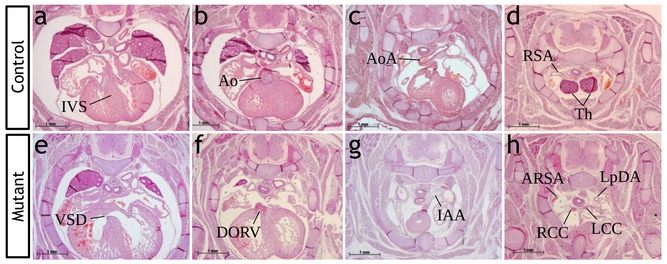

Fig 1. E15.5 embryos were embedded in wax, sectioned and stained using Haematoxylin & Eosin. **a-d,** Sections of control embryo hearts to demonstrate normal morphology. The interventricular septum (IVS), aorta (Ao), aortic arch (AoA), right subclavian artery (RSA) and thymus (Th) are indicated. **e-h,** Mutant (*Pax9*-null) embryo hearts demonstrating defects such as ventricular septal defect (VSD; **e**), double-outlet right ventricle (DORV, **f**), interruption of the aortic arch (IAA, **g**), left persisting dorsal aorta (LpDA, **h**) and aberrant right subclavian artery (ARSA, **h**). Scale, 1mm.

Defect	VSD	DORV	OA	IAA	ARSA	LpDA	LCC	Th
n	5/5	3/5	1/5	5/5	5/5	4/5	1/5	5/5
%	100%	60%	20%	100%	100%	80%	20%	100%

Table 1. Incidence of cardiovascular and thymus defects seen in *Pax9*-null embryos (abbreviations as above, plus OA, over-riding aorta; LCC, absent left common carotid; Th, absent thymus).

Measuring the aorta in control and *Pax9*-null embryos

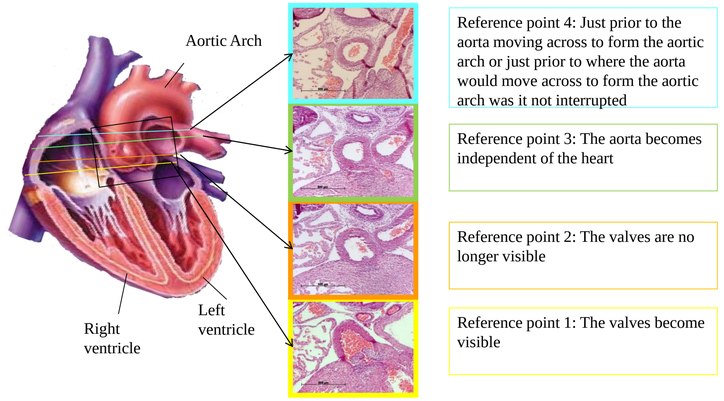

Fig 2. Representative images to illustrate the regions at which measurements of the aorta were taken from, and explanations of how the region was identified.

Results: Measuring the aorta

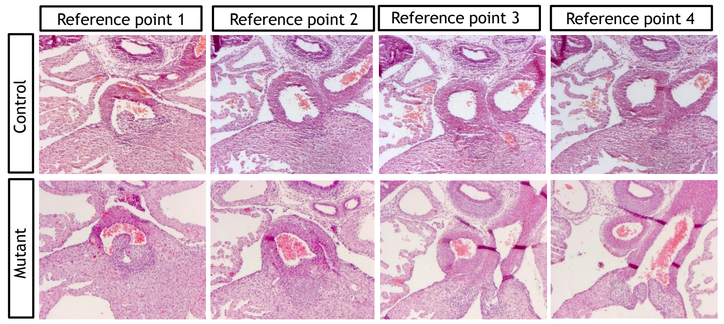

Fig 3. Representative images to show reference points at which measurements were taken from in control and mutant embryos.

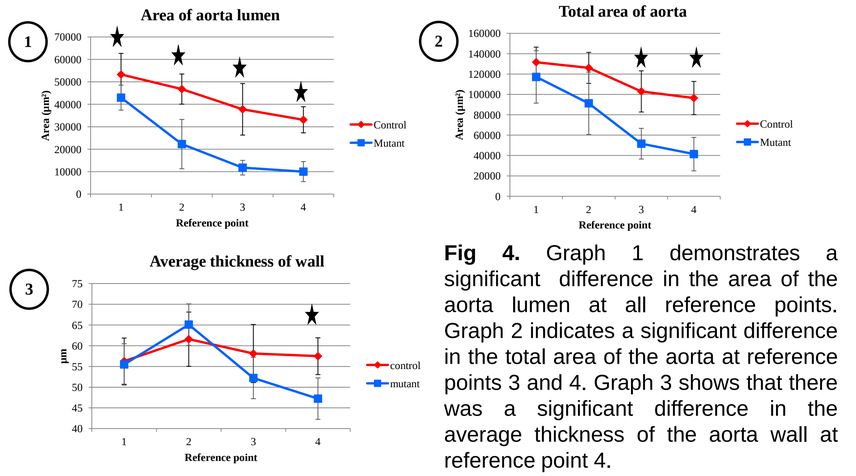

Fig 4. Graph 1 demonstrates a significant difference in the area of the aorta lumen at all reference points. Graph 2 indicates a significant difference in the total area of the aorta at reference points 3 and 4. Graph 3 shows that there was a significant difference in the average thickness of the aorta wall at reference point 4.

Bicuspid aortic valves in *Pax9*-null embryos

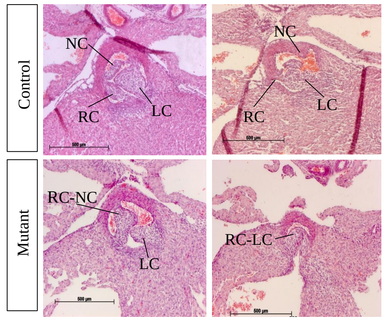

Fig 5. Representative images of the aortic valves in control and *Pax9*-null embryos.

Embryo #	Aortic valve	Comment
46.6	Bicuspid	RC fused to LC, NC not visible
68.6	Bicuspid	RC fused to LC, RC fused to NC
279.5	Bicuspid	RC fused to NC
279.8	Bicuspid	RC fused to NC
279.10	Bicuspid	RC fused to LC, NC not visible

Table 2. Bicuspid aortic valves seen in *Pax9*-null embryos. The aortic valve is bicuspid (i.e. abnormal) in all *Pax9*-null embryos studied (RC, right coronary; LC, left coronary; NC, non coronary)

Coronary artery origin in control and *Pax9*-null embryos

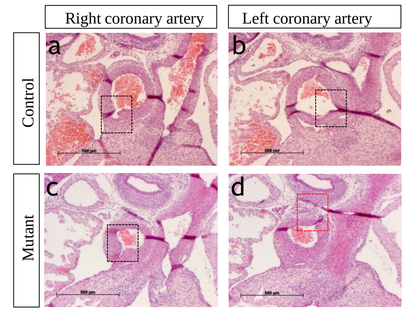

Fig 6. Representative images of the origin of the coronary arteries in control (**a**, **b**) and *Pax9*-null embryos (**c**, **d**). All embryos showed normal coronary artery origin, with the exception of one *Pax9*-null embryo which showed an abnormal left coronary artery origin (**d**).

Conclusions

1) The aorta is hypoplastic in *Pax9*-null embryos after the aorta becomes independent of the heart, the lumen of the aorta is hypoplastic at all reference points in *Pax9*-null embryos, and the average thickness of the aortic wall is only significantly thinner in *Pax9*-null embryos at reference point 4.
2) All *Pax9* control embryos have a normal tricuspid aortic valve, whereas all *Pax9*-null embryos have an abnormal bicuspid aortic valve and some type of valve fusion event.
3) All Pax9 control embryos have normal coronary artery origin, whereas the majority (i.e. 4/5) *Pax9*-null embryos have normal coronary artery origin. One embryo presented with the left coronary artery coming off the NC valve, although this could be a variation of normal.

References

Santen *et al* (2012) Further delineation of the phenotype of chromosome 14q13 deletions: (positional) involvement of *FOXG1* appears the main determinant of phenotype severity, with no evidence for a holoprosencephaly locus. *J. Med. Genet.* **49**(6): 366-72.