Validating the potential downstream targets of Pax9

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Introduction

Congenital heart defect (CHD) is the most common congenital anomaly in the United Kingdom, with a reported prevalence of 51.2 cases per 10,000 total births in 2010[1]. CHD also accounts for 26% of anomaly-related perinatal mortality rate[1]. Research in heart development— and the genetic network which governs it— is therefore important because new findings would allow possible screening for mutations in the genes involved, and this may be beneficial for prospective parents in the area of genetic counselling.

Pax9 is an important gene that is implicated in cardiac development. It is expressed in the embryo and when its function is disrupted in *Pax9*-null mice, severe heart defects develop. *Pax9* may also interact with *Tbx1* - a gene involved in DiGeorge Syndrome.

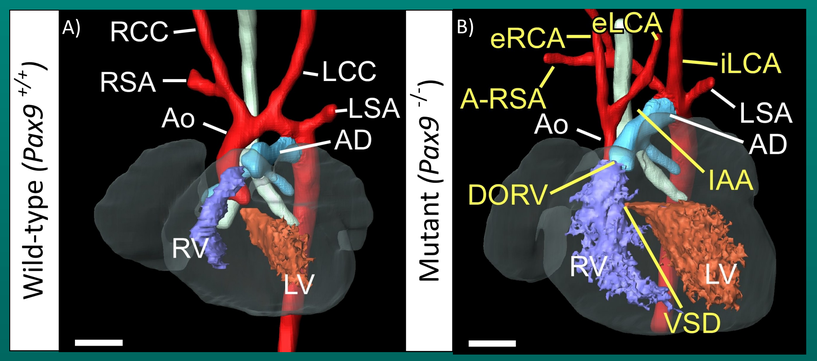

Figure 1 : A) normal heart (AD, arterial duct; Ao, aorta; LCC, left common carotid; LSA, left subclavian artery; RCC, right common carotid; RSA, right subclavian artery). B) heart from a *Pax9* $^{-/-}$ embryo with major cardiovascular defects (A-RSA, aberrant right subclavian artery; DORV, double-outlet right ventricle; IAA, interrupted aortic arch; VSD, ventricular septal defect). Scale, 500µm. *Figure provided by Dr. S. Bamforth.*

Aim and objectives

An RNA-sequencing experiment has been performed to investigate the differences in levels of gene expressions between wild-type and *Pax9*-null mouse embryos. The aim of this project was to validate the data obtained from the RNA-sequencing experiment. This will hopefully identify a genetic regulatory network involving *Pax9* that controls development of the heart and its major vessels.

Methods

- Embryos at E9.5 were dissected from pregnant transgenic mice and genotyped by polymerase chain reaction (PCR) to identify mutant (Pax9$^{-/-}$) and wild-type (Pax9$^{+/+}$) embryos.
- RNA extracted from four mutant and four control samples within the somite ranges of S22-S25 was then transcribed to produce respective cDNA libraries.
- The *Pax9* downstream targets selected for validation, based on their predicted functions and involvement in cardiovascular development, were Hist1h4m, Gpr180, Nae1, Rap2a, Emcn and Adk.
- A quantitative PCR (qPCR) experiment was performed using SYBR Green chemistry to analyse the differences in levels of gene expressions between mutant and wild-type embryos . The fold differences in gene expressions obtained from this qPCR experiment were then compared to the data obtained from RNA-sequencing.

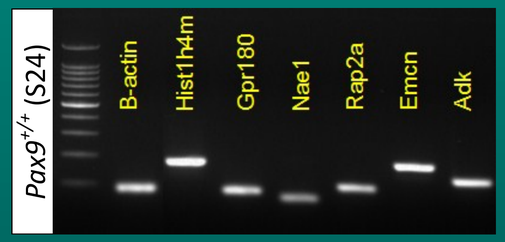

Figure 2 : RT-PCR products of E9.5 mouse embryos shown using gel electrophoresis, to ensure that the primers are working as expected.

Results

Genes of interest	Fold differences In RNA-Sequencing	Fold differences in qPCR
Hist1h4m	118.38010 (p = 0.0000000009)	2.171085 (p =0.43)
Gpr180	19.89712 (p = 0.004)	1.26181 (p = 0.37)
Nae1	6.741893 (p = 0.00007)	1.05483 (p = 0.88)
Rap2a	6.391603 (p = 0.001)	1.32980 (p = 0.39)
Emcn	5.524674 (p = 0.005)	0.91424 (p = 0.71)
Adk	4.803396 (p = 0.00005)	1.32396 (p = 0.26)

Table 1 : Comparison between the fold-differences in gene expressions of *Pax9* $^{-/-}$ (relative to that of *Pax9* $^{+/+}$) observed in the RNA-sequencing experiment and that achieved by qPCR.

Discussions

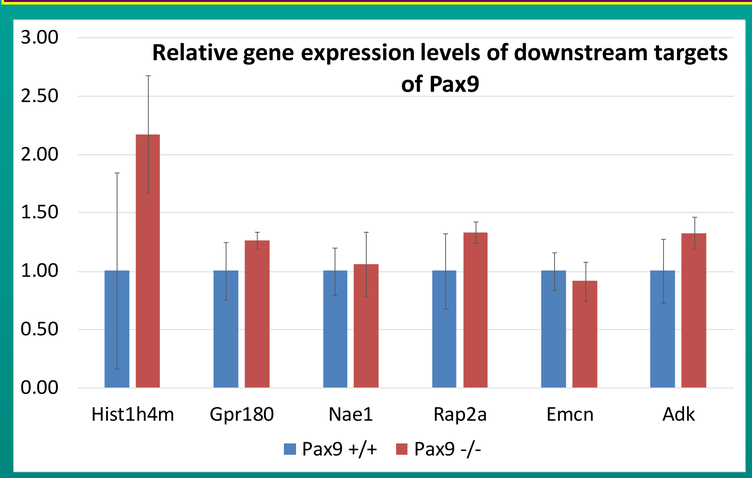

Figure 3 : Graph of qPCR results showing the levels of gene expression in *Pax9* $^{-/-}$ mice, relative to that of *Pax9* $^{+/+}$ mice.

- None of the qPCR data were statistically significant – a stark contrast to the data gathered from RNA-sequencing.
- The non-significance could be attributed to the small sample size and the variance of the data collected, as illustrated by some of the large error-bars.
- Needless to say, these gene targets need to be further validated by repeated qPCR experiments and/or by using a different method, for instance, whole-mount in situ hybridisation.

Conclusion

None of the selected targets were validated by qPCR as a differentially expressed gene. Although RNA-sequencing may be a good approach to produce a list of differentially expressed genes, it is still important to validate these data. This is to ensure that the discoveries are true especially since there is a possibility of false positives in RNA-sequencing.

References

1. Springett A. and Morris JK. (2012). Congenital Anomaly Statistics 2010 : England and Wales. London.