Molecular Cues of Heart Development

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CONTEXT

Fifteen million babies are born preterm every year [1]. These individuals have an increased risk of developing serious health conditions, as their organ systems are often undeveloped upon birth. One crucial organ affected is the heart. Therefore, it is essential to understand how the heart develops from fetal stages to adulthood in healthy individuals in order to identify differences in the heart development of those born prematurely.

INTRODUCTION

- The main contractile unit of striated muscle is the sarcomere.
- The sarcomere consists of actin and myosin filaments which slide over each other resulting in contraction.
- Regulation of muscle contraction and relaxation is implemented by troponin (Tn) [2].

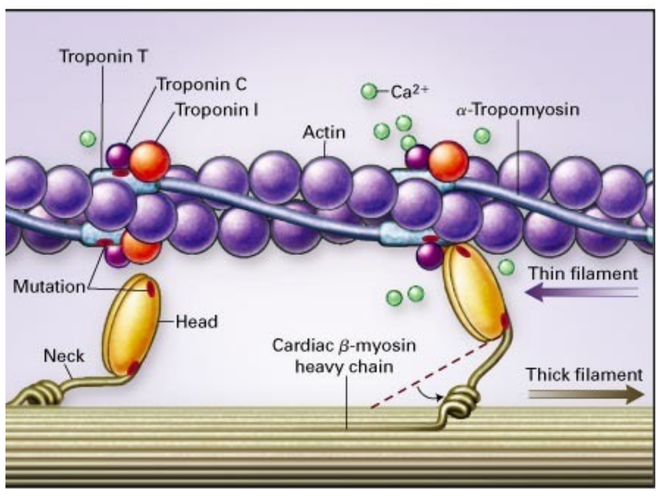
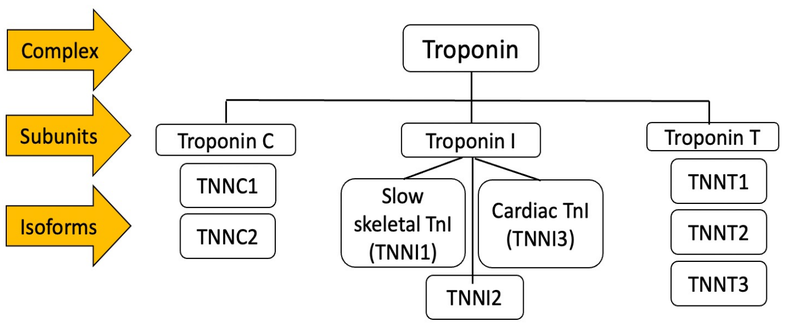


Figure 1 *– the sarcomeric unit [3]*

- Tn is made up of three subunits. Each subunit of Tn can be expressed in slightly different ways, known as isoforms indicated in the figure below.



This project will investigate two different isoforms of Tn I;
→ Cardiac Tn I – (gene name TNNI3)
→ Slow skeletal Tn I – (gene name TNNI1)

AIM

To compare the structure of TNNI1 and TNNI3 isoforms and identify their relative abundance at different stages of healthy heart development.

METHODS

Western blot

- Proteins from Cavia porcellus heart tissue were separated by SDS-gel electrophoresis. This divided the proteins according to size, with smaller proteins migrating more quickly through the gel.
- Proteins were then transferred to a nitrocellulose membrane.
- Primary antibodies (TNNI1 and TNNI3) and secondary antibodies (goat-anti-mouse) were added to allow immunodetection of specific TnI proteins present on the membrane.
- Data was quantified using intelligent quantifier software

Proteomic analysis

- Mass spectrometry data previously acquired from the laboratory [4] was analysed. This identified proteins in Cavia porcellus tissue according to their mass charge ratio.

RESULTS

Isoform-specific changes in expression between fetal and adult hearts

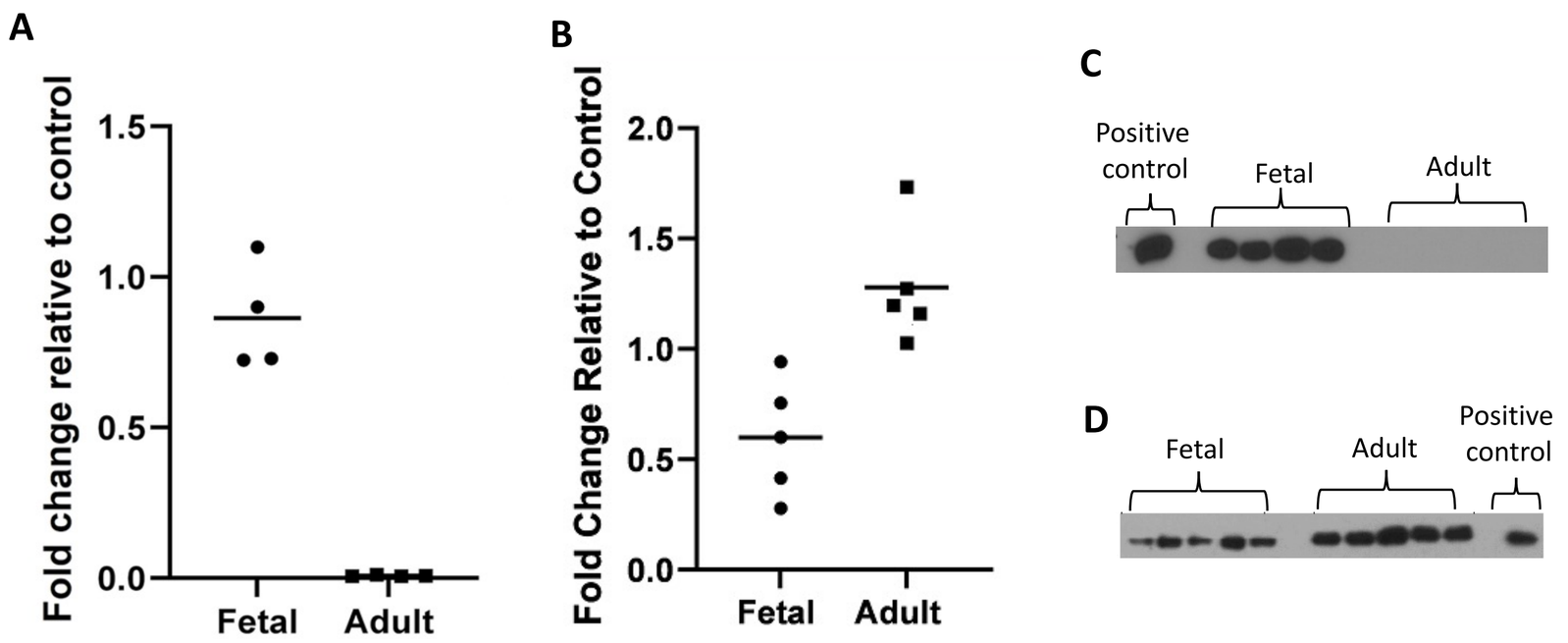


Figure 3 *– Quantification of A) TNNI1 and B) TNNI3 from western blotting (paired T-test, P<0.05). TNNI1 was more abundant in fetal samples (0.863±0.179) in comparison to the adult samples (0.008±0.002). In contrast, TNNI3 was significantly lower (paired T-test, P<0.05) in fetal samples (0.599±0.264) compared to adult samples (1.278±0.2698). The corresponding western blots for TNNI1 and TNNI3 are shown in C) and D) respectively.*

Different trajectories of TNNI1 and TNNI3 expression during heart development and maturation

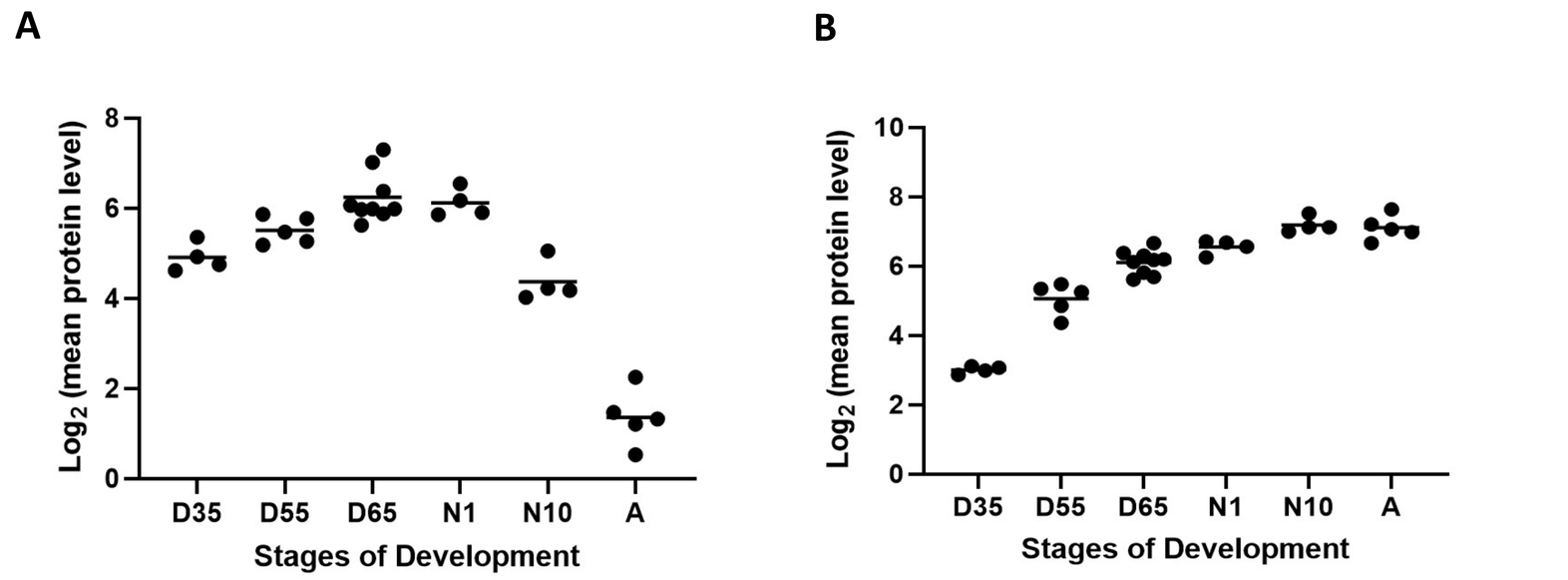


Figure 4 *– Mass spectrometry data showing changes of A) TNNI1 and B) TNNI3 protein levels over development (log_2 mean of n>4) D35,55,65- Fetal day 35,55,65. N1,N10- Neonatal day 1 and 10. A – adulthood. The protein level of TNNI1 gradually increased to 6.243±0.557 at day 65 of the fetal stage of development. TNNI1 protein levels then significantly decreased during neonatal development, to 1.356±0.617 during adulthood. In contrast, TNNI3 protein levels were lowest during early fetal development. There was a significant increase in TNNI3 by day 65 of fetal development, which continued to increase to 7.109±0.3562 during adulthood. (P<0.05, ANOVA multiple correction, followed by Fisher's least significant difference post-hoc test).*

N-terminal amino acid sequence differences evident between TNNI1 and TNNI3

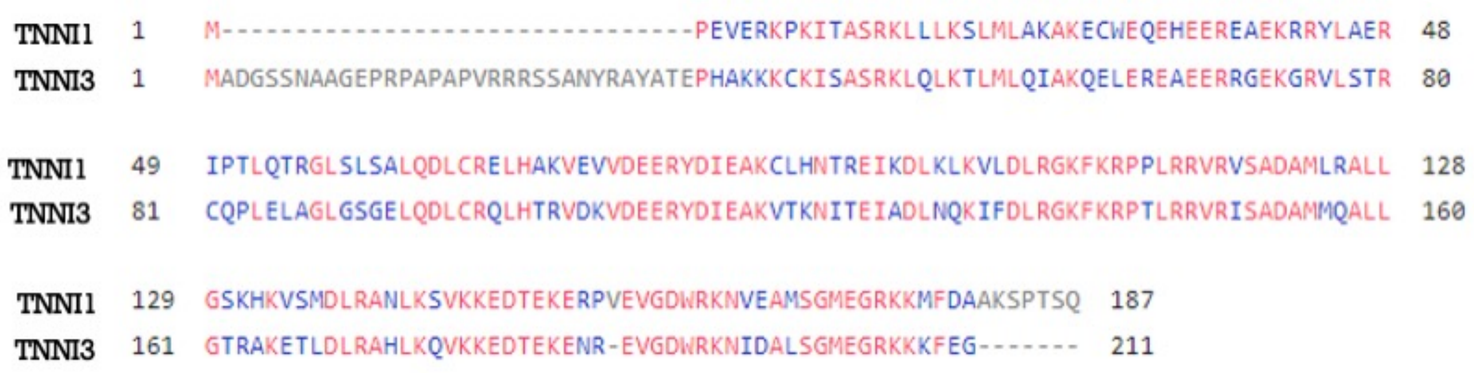


Figure 5 *– A comparison of amino acid sequences (also called primary structure) of TNNI1 and TNNI3 in Cavia porcellus. Similarities in the amino acid sequence of the two TnI isoforms are shown in red. Differences between the two sequences are shown in blue. The amino acids in grey show an insertion or deletions between the two sequences. There is a 62% similarity between TNNI1 and TNNI3 in Cavia porcellus.*

CONCLUSIONS

1. During development, the heart undergoes many changes. In fetal stages of heart development, TNNI1 is predominantly expressed. However, adult tissue has TNNI3 as the overwhelming isotype. Therefore, during development, there is a switch between these two isoforms.
2. There is 38% difference in amino acid composition between the primary structures of TNNI1 and TNNI3. These differences lead to contrasts in the functions of the two isotypes. Experimentation in mice by Solaro et al 1988, provides evidence to suggest that TNNI1 has an increased tolerance to acidic environments, and a greater calcium sensitivity, in comparison to TNNI3.
3. Future studies examining the cardiac functional implications of relative TNNI1/TNNI3 expression levels at preterm birth, is of paramount importance in order to target treatment for this population.

REFERENCES

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