Does CDKL1 regulate the development of the cardiovasculature? Developing and testing a hypothesis.

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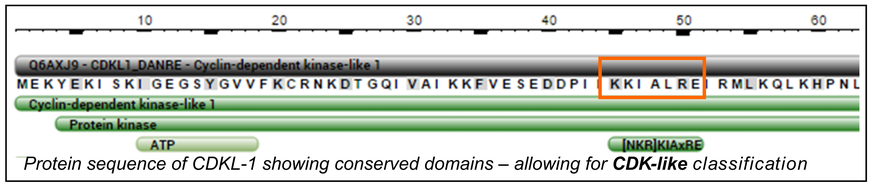
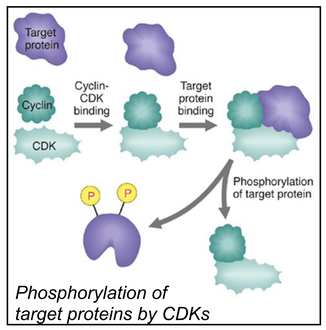
Abstract and Hypothesis.

The **cyclin dependent protein kinase** family regulates a wide range of cellular functions such as cell cycle progression, differentiation, and apoptosis. They are kinase enzymes which regulate other proteins by attaching a phosphate to serine or threonine amino acid residues in target proteins. They have two functional domains, a cyclin binding domain and a kinase domain. Cyclin binding activates these proteins mediating a cellular process via a signalling cascade. CDKL1 has been classed as cyclin dependent "like" as it contains the conserved domain for binding cyclin "KKIARLE" as well as a kinase domain. The structure of the kinase domain has been determined and is very similar to the well characterised human CDK2 (1,2).

No function has been assigned to CDKL1. Our study identified an elevated expression in response to heart attack and *In Situ* hybridisation analysis of Zebrafish indicates that CDKL1 may signal blood vessel formation. Through *Genome Expression Omnibus* searches we propose that **CDKL1** mediates this through the **Sonic Hedgehog, VEGF, NOTCH** signalling pathway.



*Protein sequence of CDKL-1 showing conserved domains – allowing for **CDK-like** classification*



Phosphorylation of target proteins by CDKs

CDK2 kinase domain(1)

CDKL-1 kinase domain (2)

CDKL1 expressed in the Zebrafish hypochord and elevated expression in hypoxic embryos.

CDKL1 expression normally decreases from 24-72hpf but it persists in the notochord (black arrow), hypochord (red arrow) and the prenephric duct (green arrow) (Figure 1).
Localisation of CDKL1 to the hypochord is significant because the hypochord plays a role in positioning and development of the dorsal aorta (3), which develops in an anterior to posterior direction. Figure 1 shows CDKL1 expression along the hypochord at 24hpf, an anterior regression at 48hpf and absence of labelling at 72hpf.
We propose that CDKL1 is involved in signalling the development of the dorsal aorta.
The effect of blood flow on CDKL1 expression was studied by stopping the heart in 48hpf embryos with the anaesthetic Tricaine. The label for CDKL1 was similar to that in untreated embryos. Embryos that develop in oxygen-deficient conditions (hypoxia) grow more blood vessels. Our preliminary data suggests that CDKL1 expression is upregulated in VHL mutant (hypoxic) embryos (3dpf). These embryos were generated from a heterozygous cross (vhl+/- x vhl +/-), noting that homozygous mutants are not viable beyond the embryo stage.
Figure 2 shows the segregation of 19 offspring with the expected phenotypic ratios of a monohybrid cross (the vhl gene exhibits codominance). The bottom right panel shows higher expression of CDKL-1 between the muscle blocks (MB) and in the notochord (N).

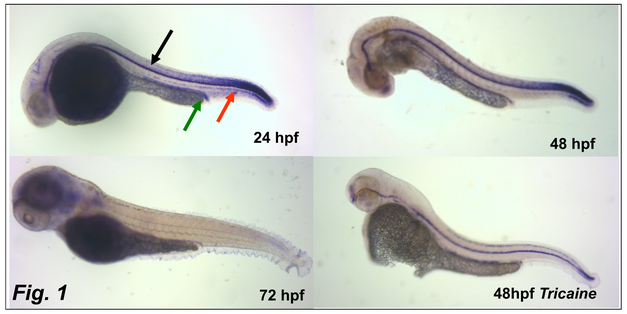
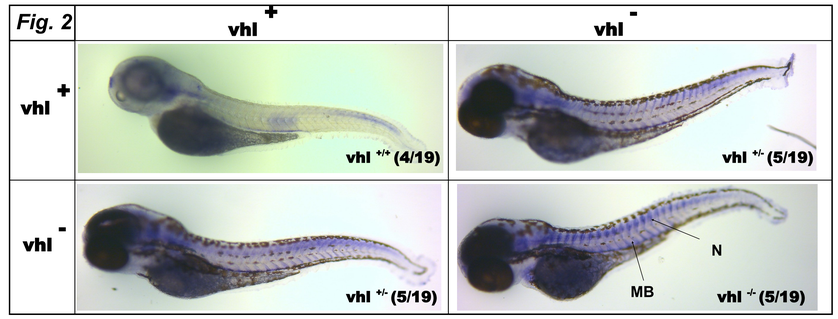
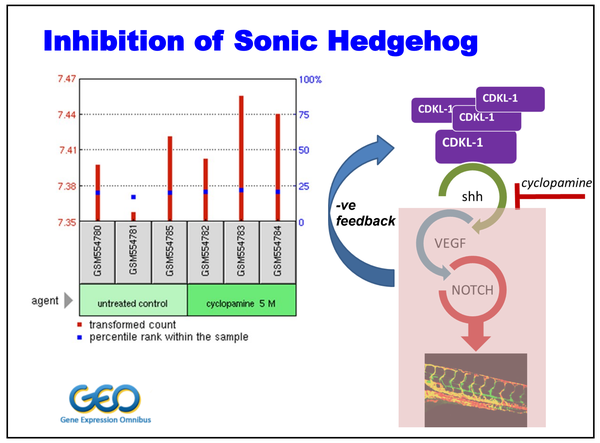


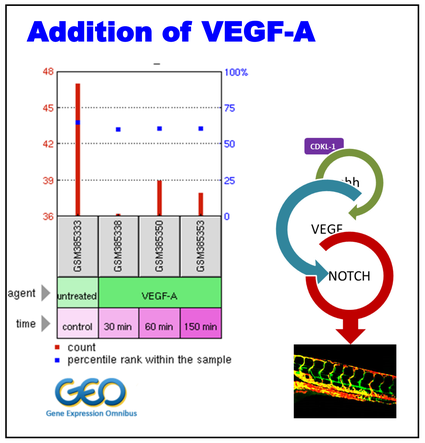
Fig. 1



Inhibition of Sonic Hedgehog



Addition of VEGF-A

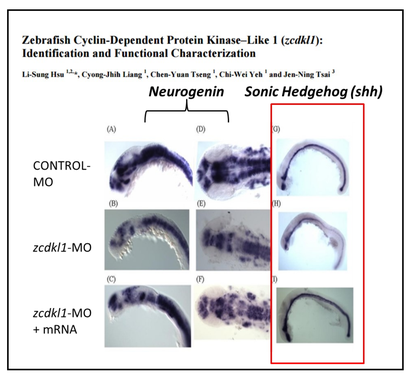


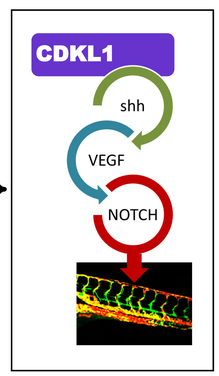
Questioning the hypothesis.

The Gene Expression Omnibus (GEO) is a public repository for high-throughput gene expression data and allows the retrieval of heterogeneous data sets from high-throughput gene expression experiments.
The two studies presented are of CDKL1 expression levels in Human Endothelial Cells in response to inhibition of SHH with cyclopamine and the addition of VEGF.
Our proposed pathway is corroborated by this data.
The elevated expression of CDKL1 in response to an inhibition of SHH can be explained via a negative feedback mechanism, whilst the addition of VEGF, which acts downstream of both SHH and CDKL1, demonstrates a reduced level of CDKL1 expression.
Our aim is to ask these questions experimentally in the Zebrafish.

Proposed mechanism: Sonic Hedgehog Regulation.

Hsu *et al.* 2011 (4) characterised CDKL1 expression in Zebrafish, they performed morpholino reverse genetics analysis on CDKL1. Removal of CDKL1 resulted in a reduced expression of Sonic Hedgehog (SHH), rescue with CDKL1 mRNA elevated SHH relative to the control. This data suggests that CDKL1 has a regulatory role over the gene expression of SHH.
Sonic Hedgehog is a morphogenic protein that is involved in embryonic development and the regulation of adult stem cell differentiation. Importantly, its role in **Arterial Endothelial Differentiation** has been widely reported. (5)





Future Work: Experimental Questions

Pharmacological Inhibition.
1.Does SHH inhibition (cyclopamine) alter CDKL1 expression?
2.Does VEGF inhibition (AV951) alter CDKL1 expression?
3.Does NOTCH inhibition (DAPT) alter CDKL1 expression?

Zebrafish Mutants.
1. Does NOTCH inhibition in the Mind Bomb (Mib) mutant alter CDKL1 expression? Mind Bomb is a ubiquitin ligase that positively regulates NOTCH signalling.
2. What is the effect of blood flow on the expression of CDKL1? Tnnt2– This protein is necessary for the contraction of cardiomyocytes, the mutant has no blood flow.

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References

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3) Development of the hypochord and dorsal aorta in the zebrafish embryo (Danio rerio). Eriksson, J. and Löfberg J. J. *Morphol 2000 244(3): 167-176.*

4) Zebrafish cyclin-dependent protein kinase-like 1 (zcdkl1): identification and functional characterization. Hsu LS[1], Liang CJ, Tseng CY, Yeh CW, Tsai JN. Int J Mol Sci. 2011;12(6):3606-17.

5) Sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. Lawson ND[1], Vogel AM, Weinstein BM. Dev Cell. 2002 Jul;3(1):127-36.