The novel CDKL-1 gene *potentially* signals Development of Cardiovasculature

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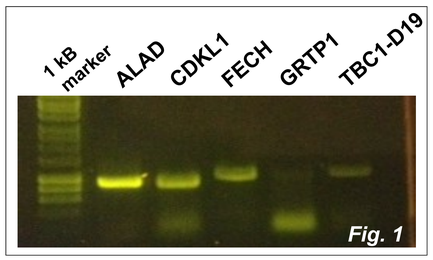
Abstract:

Cardiovascular disease (CVD) is the single largest cause of death in the UK. It accounts for around 200,000 deaths a year, mostly as a result of heart attack or stroke. Arterial blockage (or occlusion) is a major factor. We aim to characterise novel genes that are differentially expressed in a clinical analysis of patients who have suffered a heart attack by studying orthologous genes in the Zebrafish cardiovascular system. These genes may be important in the response to a heart attack or act as biological markers for heart disease. We analysed a list of 82 genes and selected ALAD, CDKL1, FECH, and TBC1D19 for further study. Preliminary data for Cyclin-Dependent Kinase-Like 1 (CDKL1) show that in 24-48hpf embryos it is localised to the hypochord, a precursor to the dorsal aorta. Its expression pattern changes in a model of hypoxia, in which blood vessel formation is stimulated. These results suggest that CDKL-1 may mediate signalling for blood vessel formation, which is essential for recovery following cardiovascular failure.

Methods: Identifying novel candidate genes .

The 82 genes identified from patients were ranked in terms of their differential expression and temporal expression profiles over the 90 day test period. Functional information was derived from The National Center for Biotechnology Information and protein sequence alignments to identify Zebrafish orthologs were performed using Ensembl (Figure 1). We selected genes with greater than 70 % homology and then looked for candidates which had novel or poorly characterised functions.

Gene	Function	Rank	% identity	Literature	*In-situ*
CDKL1	cyclin-dependent kinase-like 1	1	77	Zebrafish cell cycle progression, differentiation, and apoptosis	Yes
MEIS1	Meis homeobox 1	2	92	Development endothelial cells Zebrafish	Yes
FECH	ferrochelatase	15	70	enzyme in haem biosynthesis pathway	none
MNAT1	menage a trois homolog 1,	26	74	Acts to assemble CDK7 and cyclin H .	none
TBC1D19	TBC1 domain family, member 19	29	77	*no known use- may act as GTPase activating protein	none
ALAD	aminolevulinate dehydratase	34	73	Haem synthesis, platelet expressed proteins associated with CVD	none
DDX19B	DEAD (Asp-Glu-Ala-Asp) box polypeptide 19B	36	78	Export of mRNA from nucleus - mutagenesis limits neuronal development in Zebrafish	none,
SOX6	SRY (sex determining region Y)-box 6	42	76	Chondrocyte differentiation pathway and leads to activation of collagen gene	none
SFRP2	secreted frizzled-related protein 2	46	72	Development of the nervous systen	Yes
UBE2H	ubiquitin-conjugating enzyme E2H	54	98	Acts on histones and cytoskeletal proteins involved in the degenerative pathway of the motor neurones -	nine
EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa	55	78	Associated with testicular germ cell tumor - nucleic binding	none
AK1	adenylate kinase 1	58	74	Huntington's disease, methylmercury exposure	Yes



PCR, cDNA analysis.

We used PCR to confirm expression of candidate genes in Zebrafish embryos and selected those that are novel (TBC1-D19 (2)), exist in the same biochemical pathway (ALAD & FECH) or are important developmentally (CDKL1).

Figure 1. cDNA derived from mRNA extracted from 48 hpf embryos was analysed with PCR primers to a 1000 bp region of the Zebrafish orthologs. Amplification of these regions from 4 of the candidate genes indicates their expression in the embryo and makes them viable for In-situ Hybridisation. This is a technique which we can regularly perform with increasing reliability in our school laboratory.

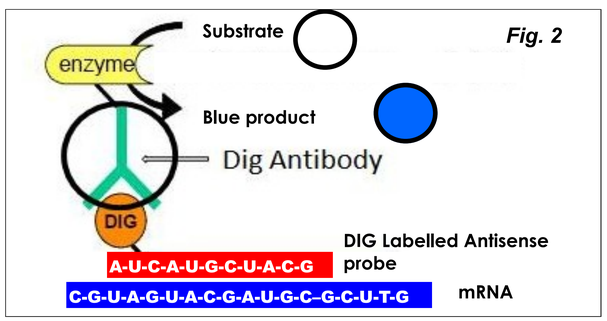


Fig. 2

In-situ hybridisation

This identifies gene expression patterns in the fish embryo. Cells expressing a gene will contain mRNA, which can be identified with antisense RNA probes that are subsequently detected by immunohistochemistry with an alkaline phosphatase-conjugated antibody against digoxigenin and a chromogenic substrate (Figure 2) (2)

Results: Gene expression of candidate genes.

Figure 2: Whole mount In-Situ hybridisation of Wild Type 2dpf embryos figure 2 . Embryos were labelled with antisense DIG RNA probes for the four candidate genes. Immuno-staining was inconclusive for ALAD, FECH and TBC1-D19, the staining most likely endogenous alkaline phosphatase activity in the notochord. The CDKL-1 probe, however, localised to distinct structures of the embryo, including the notochord and hypochord.

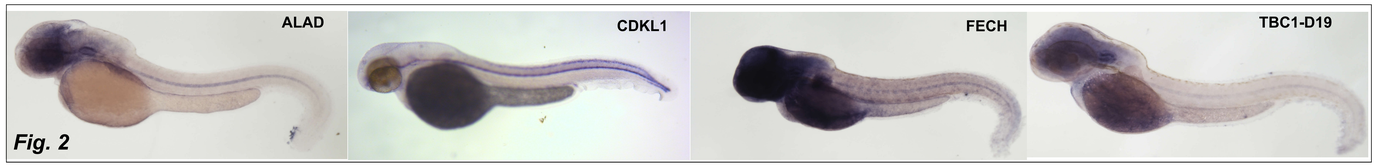


Fig. 2

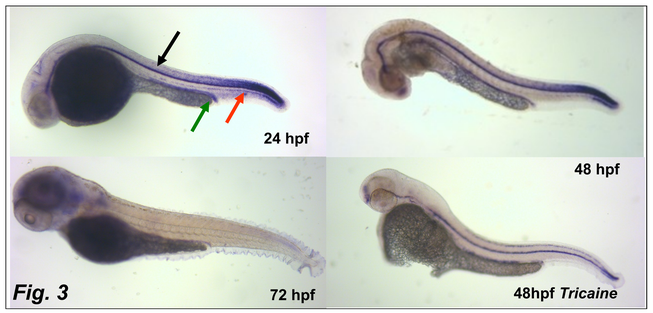


Fig. 3

CDKL-1 is expressed in the hypochord.

CDKL-1 expression normally decreases from 24-72 hpf but it persists in the notochord (black arrow), hypochord (red arrow) and the prenephric duct (green arrow) (Figure 3). Localisation of CDKL-1 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 3 shows CDKL-1 expression along the hypochord at 24hpf, an anterior regression at 48 hpf and absence of labeling at 72hpf.

We propose that CDKL-1 is involved in signalling the development of the dorsal aorta. The effect of blood flow on CDKL-1 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.

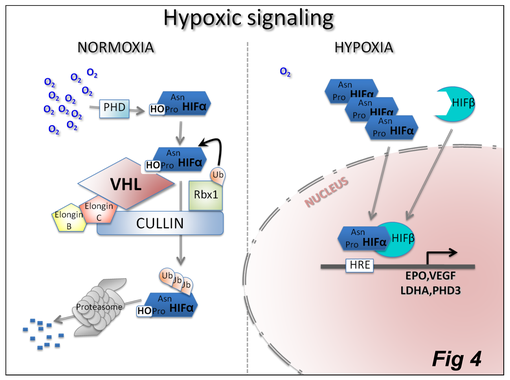


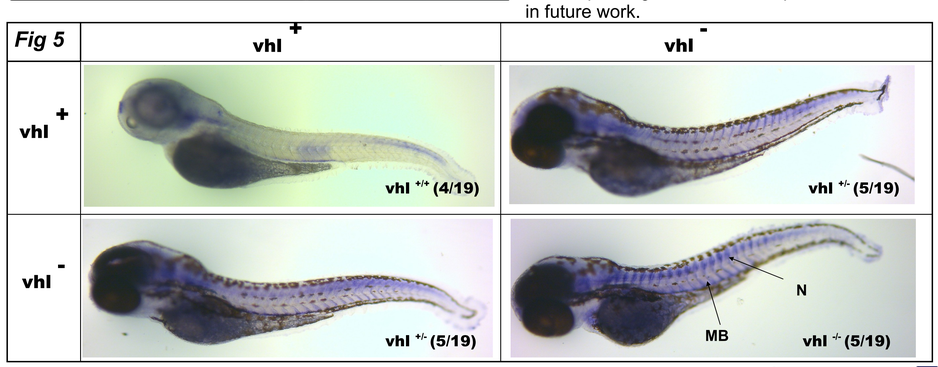
Fig 4

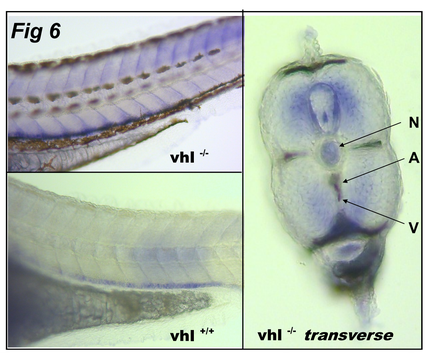
CDKL-1 expression in hypoxic embryos : Evidence for a role in vasculature development.

Embryos that develop in oxygen-deficient conditions (hypoxia) grow more blood vessels. Hypoxia can be mimicked in Zebrafish through the activation of the HIF transcription factor complex, which regulates angiogenesis via agents such as Vascular Endothelium Growth Factor (VEGF). VHL targets HIF for breakdown and repression of VEGF signalling and its deletion leads to embryos with increased blood and vasculature.

Our preliminary data suggests that CDKL-1 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 5 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). Figure 6 compares higher magnification images for mutant vhl (top) and WT (bottom). The transverse section shows staining in the main axial aorta (A) and vein (V) with diffuse staining in the somatic tissue which may be signalling angiogenesis.

Our results have led us to the hypothesis that CDKL-1 has a novel role in development of blood vessels, explaining its increased expression in heart attack patients. We plan to take this hypothesis forward in future work.





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