Identifying novel genes in Cardiovascular Disease.

Tapton SCHOOL

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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 in CVD. Blockage may occur when a blood clot obstructs the flow of blood in an artery.

Our ***project aims*** to identify novel genes involved in CVD. A Clinical study identified 82 genes with significant differences in their expression levels as a result of a heart attack. We are looking for the expression pattern of novel gene orthologues in the Zebrafish model organism using ***in-situ* hybridisation**. Any genes we locate to the cardiovascular system could play a part in the response to a heart attack, or may serve as future biological markers for heart disease.

In-situ HYBRIDISATION

Our method identifies where genes are expressed in the body of the fish embryo. Cells expressing a gene will contain mRNA which has been transcribed from the DNA. It is possible to amplify Antisense RNA probes. This probe will hybridise specifically to the mRNA which is then detected by immunohistochemistry using an alkaline phosphatase-conjugated antibody against digoxigenin and a chromogenic substrate (**Figure 2**) (2).

We have successfully repeated this assay in our school laboratory (**Figure 1**), using a probe for **KLF2a** expression in a Zebrafish (48hpf).

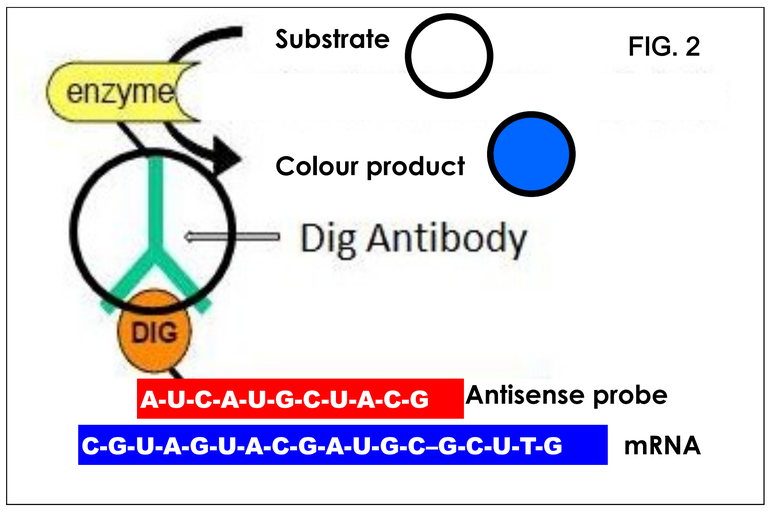

FIG. 2

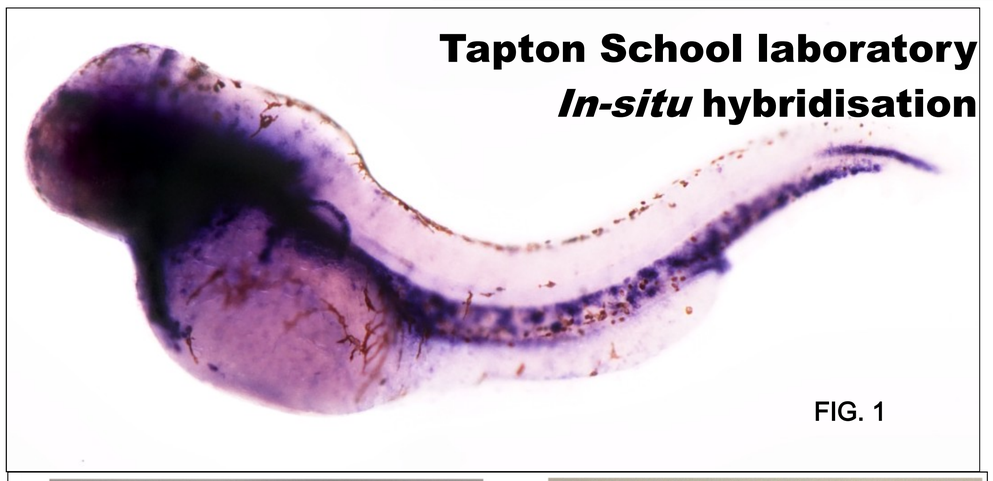

FIG. 1

KLF2

KLF2 is a zinc finger transcription factor that plays an important role in endothelial biology. Importantly, its expression in endothelial cells is regulated by blood flow. In the areas of a non-turbulent laminar flow (straight parts of arteries) there is a high level of KLF2 expression, but at the areas of branching points blood flow is turbulent and KLF2 expression is inhibited . Atherosclerosis occurs mainly in the branching points of arteries - where KLF2 expression is low. KLF2 is therefore interesting for its anti-atherogenic potential.

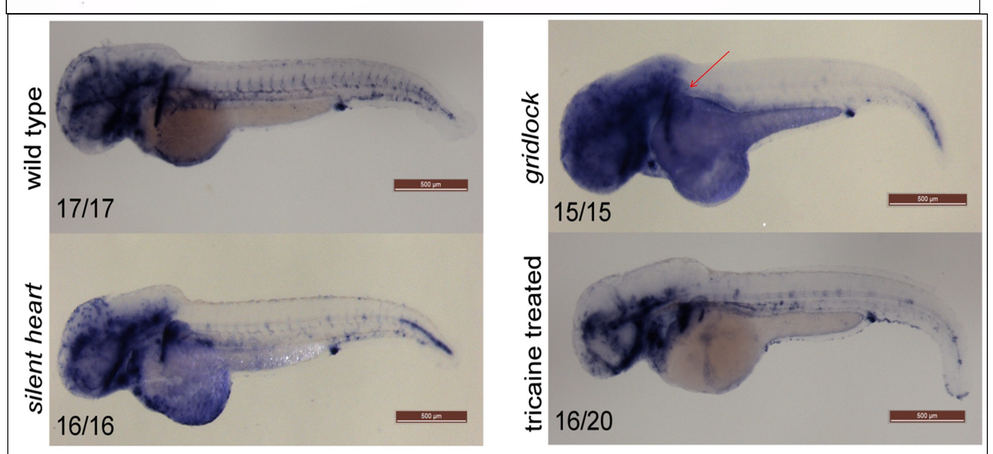

FIG. 3

Figure 1. shows the *in-situ* hybridisation performed at Tapton School. This embryo (48hpf) was treated with Lovastatin, a drug known to increase KLF2a (*KLF2 zebrafish orthologue*) expression.

Figure 3. Expression patterns are similarly demonstrated in wild type embryo (48hpf). Also shown are embryos where blood flow and hence KLF2a expression are reduced. Prevention of blood flow in developing embryos, either by specific *troponin t2* knockdown (*silent heart* morphants which never experience blood flow and survive due to oxygen diffusion for up to 7 days), in *gridlock* mutants (proximal occlusion of aorta indicated by **red arrow,**) or pharmacologically (treatment with a local anaesthetic Tricaine) show abrogated vascular expression of KLF2a *(P. Novodvorsky)*

IDENTIFYING NOVEL CANDIDATE GENES

The 82 genes identified from the clinical study were ranked in order of differential expression as well as the time that these differences were detected over a 90 day test period, (*rank 1 being the most persistently different)*. We used **Bioinformatics** to select genes for study. Protein sequence alignments between the Human and Zebrafish orthologues were performed using **Ensembl** (Figure4) . Genes with greater than 70 % homology will form the first stage of our in-situ screen (Table). In addition regions of the Zebrafish and Human chromosomes were compared to identify conservation (**Figure 5**).

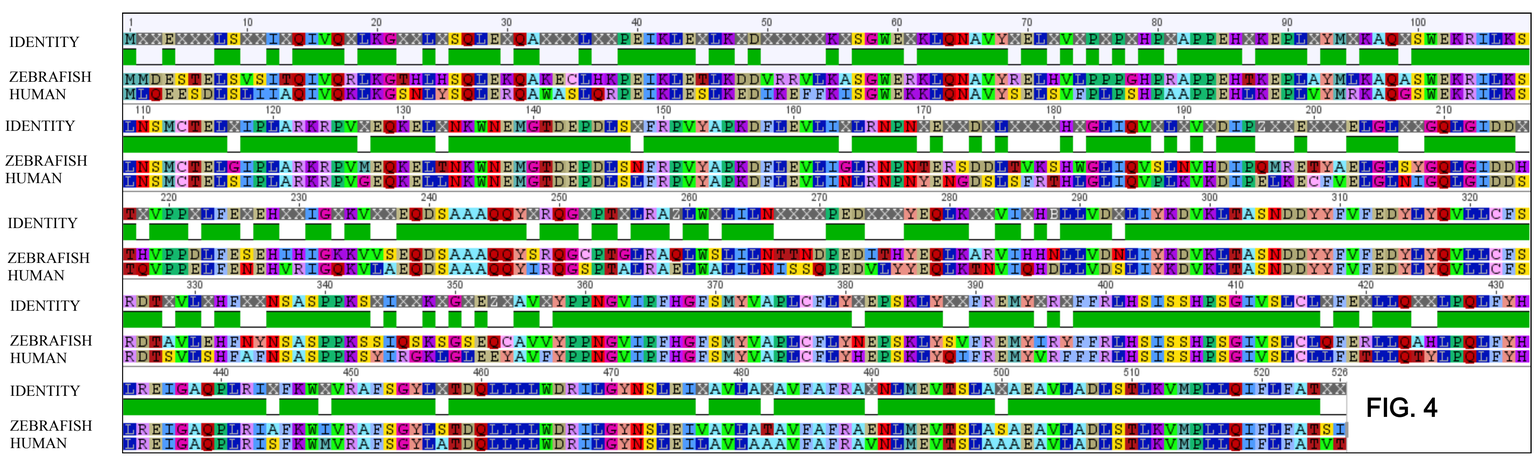

FIG. 4

Gene	Function	rank	% identity	Literature	*In-situ*
CDKL1	cyclin-dependent kinase-like 1	1	77	cell cycle progression, differentiation, and apoptosis: in Zebrafish	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, cyclin H assembly factor	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 -	Yes-
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

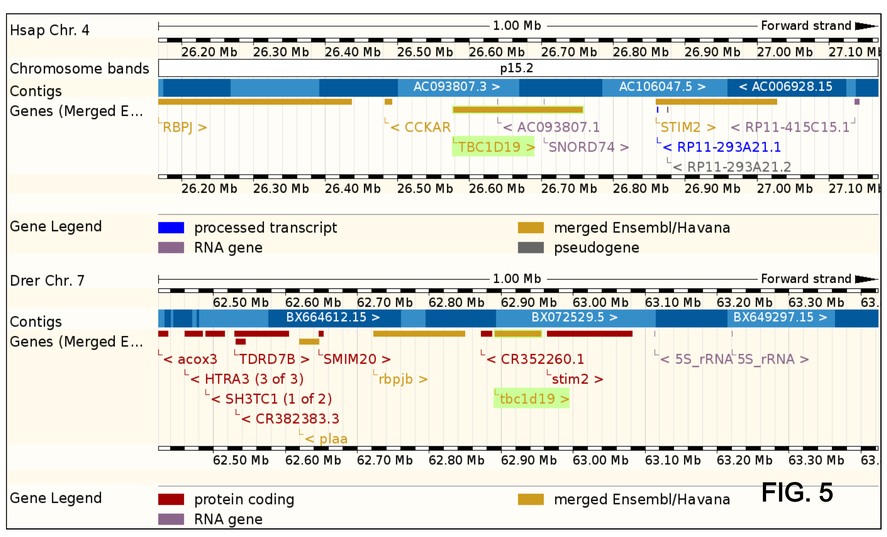

FIG. 5

CDKL1 and **MEIS1** were ranked 1 and 2, respectively. They are well conserved between fish and man, however they have already been well characterised in Zebrafish in terms of function and expression patterns in embryo development. More interesting is **TBC1D19** which was identified as having differential expression in the first three days after a heart attack, has a high protein sequence homology with the Zebrafish orthologue (77%, **Figure 4**) and a conserved chromosomal region. To date TBC1D19 has not been subject to any *in-situ* hybridisation analysis and little is known about its function. The TBC family of proteins have a conserved protein motif consisting of approximately 200 amino acids which is present in all eukaryotes. A proposed function is as a GTPase activating protein in **second messenger cell signalling** (3). We will now perform *in-situ* hybridisation analysis to locate its expression, and use similar criteria to select and screen for other candidate genes.

kiaa1109 -another approach.

kiaa1109 was identified as an evolutionarily conserved gene with no known function. Genome wide association studies **(GWAS)** have implicated variants in the chromosomal region encoding kiaa1109 with a number of human diseases, including **rheumatoid arthritis, inflammatory bowel disease and celiac disease**.
In Zebrafish kiaa1109 loss-of-function induced by morpholino antisense caused a reduction of endothelial cells in the hindbrain and forebrain, in association with **loss of vascular integrity and cerebral haemorrhage** (3).
This loss of function phenotype is rescued by treatment with the small molecule GS4012, which is known to stimulate the vascular endothelial growth factor-induced pathway **VEGF** (Figure 6). This result suggests that kiaa1109 is involved in vascular integrity and performs this function by interacting through the VEGF pathway.
We aim to identify any protein-protein interactions that occur between kiaa1109 and VEGF pathway proteins using the **Yeast 2 Hybrid system** (4).

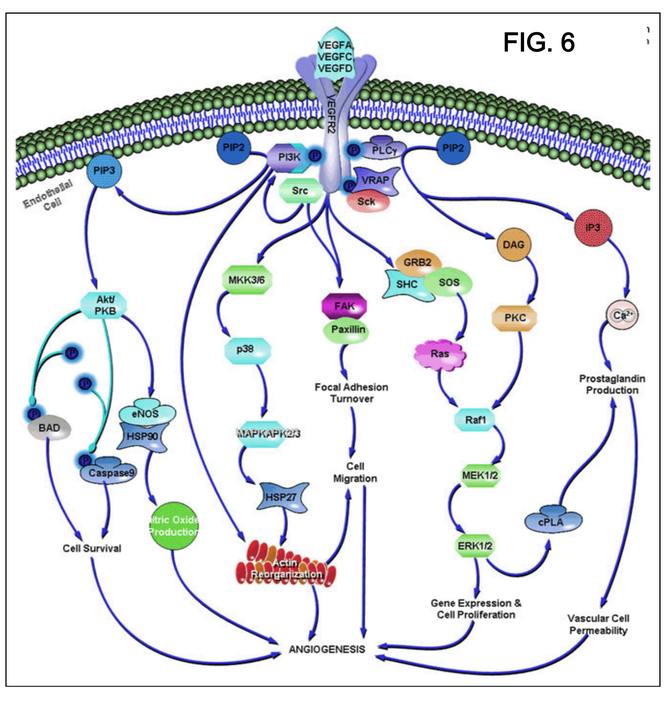

FIG. 6

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