H4

An imaging study of the neuronal subsets in the green fluorescent protein (GFP-M) line of transgenic mice

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Aims: The aim of this study was to characterise the expression of green fluorescent protein in different neuronal subsets of the Thy1-GFP-M line in transgenic mice in order to establish which populations of neurons, in an identifiable brain structure, can be studied using this line.

Methods: Coronal cerebral and cerebellar slices (400Êm), sagittal cerebellar slices including brainstem and spinal cord (400Êm) and horizontal retinal slices (250Êm) were prepared from the Thy1-GFP-M mice. Care was taken to retrieve as many slices as possible so as to preserve the histology of the prepared sections. The slices were fixed for 1hr and cryoprotected overnight. 60Êm slices were then prepared by a vibroslicer in cold PBS, pH7.2 and visualized at 488nm using a confocal microscope (Bio-Rad MRC1024). Images were acquired under both low and high magnification using 10x and 40x air Nikon lenses. Maximum intensity projections were acquired through a Z-stack set at an interval of 1Êm using a 60x oil immersion Nikon lens.

Results: The M-line shows good expression of the GFP in the mossy fibres of the cerebellum and in spinal cord axons. There was very sparse, yet intense expression that allowed visualisation of dendritic arborisation including spines in pyramidal cells of layer 5 of the cerebral cortex. There was also some neuronal expression in the hippocampus and in retinal ganglion cells. There was minimal GFP expression in the corpus callosum.

Conclusions: Since there is no record in the literature as to whether there is expression of the GFP in the corpus callosum, our observation shows that the expression of the protein in the corpus callosum is minimal and therefore it is not ideal as a model for the study of white matter injury in the brain. Due to the heterogeneous distribution of GFP together with high intensity expression in individual neurons, Thy1-GFP-M mice are suitable for anatomical and functional studies of dendritic spines in experimental models of neuronal plasticity, cellular pharmacology and learning and behaviour. This line also has the potential to be used in animal models of spinal cord injury and in regeneration experiments involving axonal sprouting and path finding.

H5

Candidate molecular regulators of developmental globin gene switching

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Aims: The specific objectives were to identify those cognate molecules that control the developmental globin gene switch from gamma to beta globin gene expression during the transition from foetal to adult erythropoiesis with advanced molecular biology tools in a Maltese family-based study.

Methods: A rare and unique Maltese family consisting of 10 family members with high levels of foetal haemoglobin (HbF ~3.5% to 20%) together with the HbF variant; Hb F Malta I [alpha2Ggamma2 117(G19)His>Arg] + Hb Valletta [alpha2beta2 87(f3)Thr>Pro] and 19 family members with normal levels of foetal haemoglobin (HbF <1.0%), spanning 4 generations was identified from testing Programs done at the Laboratory of Molecular Genetics, University of Malta in conjunction with the Thalassaemia and Molecular Genetics Clinic, Mater Dei Hospital, Malta. Extensive molecular haplotyping and DNA sequencing across the beta globin locus was carried out as explained in http://www.ithanet.eu/mutation/LabProtocols.aspx. Human Erythroid Progenitor cells were isolated and cultured from 30mls of peripheral whole blood from 15 members of the family.

Results: The Blood picture from HPFH individuals showed acanthocytosis, polychromasia, and poikilocytes. Whole genome association studies showed two independent high Lod Scores were obtained on chromosomes 19p13.2 and 15q15.5. Candidate genes that are part of the linkage block are currently being investigated by DNA sequencing. Quantitative RNA expression profiling obtained from cultured human erythroid progenitors showed a number of significantly differentially expressed genes that are strikingly associated with Haemoglobin synthesis and metabolism as well as red blood cell membrane and integrity. A list of genomic sequences that appear to be tightly linked and involved in globin gene switching has emerged from this study and are being currently validated in human cellular models.

Conclusions: The translation of studies between the in vivo phenotypes and genotypes of the family members and the in vitro quantitative expression of human erythroid progenitors provided a singular opportunity to identify the critical complexes that control switching and the order in which they are assembled into regulatory complexes together with the possibility of targeting the critical steps for bio-therapeutics by reversed switching.

H1

Personality, stress and spiritual coping of nursing students

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Aims: This correlational study is part of a longitudinal study conducted at the Institute of Health Care in Malta. It sought to identify relationships between personality, academic/ professional stress and spiritual coping of undergraduate nursing students (n=115).

Methods: Two cohort groups of students undertaking the second year Diploma Nursing/Midwifery (n=70) and BSc(Hons) (n=45); male(n=23), females(n=92); aged (18-20) years. Three self-administered questionnaires were completed in class under supervision:

NEO Personality Inventory (Costa & McCrae 1992) Academic and Professional Stress Questionnaire (Rhead 1995) Spiritual Coping Strategies Scale (Baldacchino 2002)