TrainMALTA Networking Event

25th – 26th April 2016

University of Malta Valletta Campus

TrainMALTA Partners:

University of Malta
University of Cambridge
Katholieke Universiteit Leuven

TrainMALTA Project
Faculty of Health Sciences
University of Malta

trainmalta@um.edu.mt

www.um.edu.mt/project/trainmalta

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Programme

Venue: University of Malta Valletta Campus

Monday 25th April 2016

09:30	Registration and uploading of presentations
10:30	Coffee
11:00	Welcome and Introduction Opening of the TrainMALTA Networking Event 2016 Introduction to the project Findings of Bioinformatics Needs Survey **Rosienne Farrugia**
11:30	Overview of Bioinformatics Training **Gabriella Rustici** The same but different: epigenomic landscapes of erythropoiesis and megakaryopoiesis **Luigi Grassi**
12:30	Lunch
13:30	Keynote Speech **Hana Lango Allen**
14.45	UoM presentations - **Group 1**
15:30	Coffee
16:00	UoM presentations - **Group 2**
16:30	General Discussion
17:00	End of Day 1
20:00	Social Evening – Dinner (see annex for details) Da Luigi, Rabat (Mdina)

Tuesday 26th April 2016

09:30	Platelets to study bleeding and thrombosis but also neurological syndromes **Kathleen Freson**
10:00	UoM presentations - **Group 3**
10:30	Coffee break
11:00	UoM presentations - **Group 4**
11:30	General Discussion
12:15	Closing Address **Willem H. Ouwehand**
12:30	Lunch

University of Malta Presentations
Speakers

Group 1 – Monday 25th April 14:45 – 15:30

Genetic Variation in the Maltese Population
Joanna Vella

The Malta NGS Project: The Application of High Throughput Sequencing for the Identification of Mutations in Rare and Common Diseases
Rosienne Farrugia
Julia Camilleri
Francesca Borg Carbott

The Maltese Acute Myocardial Infarction Study
Stephanie Bezzina Wettinger

Variants Effecting Inflammatory Expression Profiles Identified by a Novel Biological Pathway Approach.
Ritienne Attard

Group 2 – Monday 25th April 16:00 – 17:00

A Fruitful Fly Forward in Drug Screening for Neurodegenerative Diseases
Michelle Briffa

Generation of Stress Triggered Pluripotency of Stem Cells – A new approach to cellular medicine
Ila Tewari

Group 3 – Tuesday 26th April 10:00 – 10:30

The Genetics of osteoporosis and fragility fractures in Malta
Melissa Formosa

Rare Kidney Diseases in Children
Esther Zammit

The quest of computer scientists to discover new drugs
Jean Paul Ebejer

Group 4 – Tuesday 26th April 11:30 – 12:00

Study of the control of Human Haemoglobin gene expression using a unique Haemoglobin variant
Alexander Camilleri

A multi-omic approach to understand developmental globin gene switching
Laura Grech

Abstracts

The same but different: epigenomic landscapes of erythropoiesis and megakaryopoiesis

Luigi Grassi

Department of Haematology, NHS Blood and Transplant Centre, University of Cambridge

Red blood cells and platelets are anucleated cells responsible for oxygen transport and homeostasis, respectively. They are among the major components of blood and are formed in the bone marrow by residing progenitors that differentiate to erythroblasts (EB) and megakaryocytes (MK). Their clearly distinct phenotypes are not reflected by different transcriptional programmes. Indeed, EB and MK share a large part of their transcriptome and this is due to a largely overlapping set of transcription factors. We integrated gene expression analysis with chromatin epigenetic states and long range DNA interactions. Our analysis led us to the discovery of cell type specific 3D gene network landscapes responsible for the unique functional identities of these cells.

Platelets to study bleeding and thrombosis but also neurological syndromes

Jessica Herremans, Benedetta Izzi, Manisha Padmakumar, Anouck Wijgaerts, Kathleen Freson

Deparment of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Belgium

Platelets that control the balance between thrombosis and hemostasis play a pivotal role in cardiovascular disease and their number, volume and function are strongly heritable. Megakaryopoiesis is a very complex process that is tightly regulated by different intrinsic and extrinsic molecular mechanisms to finally form platelets. Mutations in genes that are essential for this process or for platelet function can result in an Inherited Platelet Disorder (IPD). IPDs are characterized by a high degree of genetic heterogeneity and are considered as rare diseases that are still largely unresolved. In addition to classical IPD cases that present with clinical bleeding or thrombosis problems, we study patients that only have subclinical platelet defects in combination with phenotypes such as bone (collagen-related disorders), neurological (autism or mental disability), or immune (Roifman) defects. Studies in these patients are beyond the state-of-the-art but we believe that these studies will deliver novel insights in platelet biology in addition to other disease aspects. Using NGS and DNA methylation analysis, genes and pathways will be discovered that place pointers at new regulators of platelet function and/or formation. I will discuss examples of functional genetics using *in vivo* zebrafish model and *in vitro* megakaryocyte differentiation assays from inducible pluripotent stem cells obtained from patients' fibroblasts and blood-derived hematopoietic stem cells, are next essential to characterize the role of novel candidate genes.

Genetic Variation in the Maltese Population

Joanna Vella

[1]Department of Physiology & Biochemistry, Faculty of Medicine & Surgery, University of Malta; [2]The Malta BioBank, Centre for Molecular Medicine & BioBanking, University of Malta;

Background: The Mediterranean basin has a rich demographic history. Malta saw the settlement of Sicilian's and Italian's from Calabria, together with an influx of other population groups over the years which caused a population admixture. Malta being a small island with a population of approximately 420,000 could have a very different genetic population distribution from other groupings in the same geographical region. The Maltese are not an entirely homogenous population due to founder effects which have shaped the local gene pool. Locally, very little research involving genetic bio-markers to study population variation has been carried out previously. A study of the Y chromosome to assess Mediterranean genetic structure, which included 90 Maltese samples, revealed that Malta forms part of the Central-East Mediterranean grouping with Southern Italy, Turkey and Cyprus in the same category. Another study by Zalloua *et al*. describes Maltese ancestors as Phoenician. This study included Y chromosome typing of 187 Maltese samples for which there is no reference as to how samples were collected. Autosomal short tandem repeat (STR) analysis involving 157 Maltese samples showed a close genetic relationship of the Maltese with the Sicilian population. Genetic similarity can be due to geographic position or language phylogeny. Semitic speakers have North African origin. Based on local haemoglobin quantitative epidemiological research it is hypothesised that 2-3 alleles at two loci could generate a broad range of quantitative complexity in phenotypic variation. This could account for trans-selective pressures on the regional shaping of genomes. This hypothesis will be explored using a set of lineage markers.

Objective: To investigate the effects of population migratory events in the Mediterranean and the effects of selective pressures on the genetic sub-structure of the Maltese population.

Aims:

1) To generate a genealogical profile of the Maltese population using mitochondrial DNA (mtDNA) and Y chromosome markers.
2) To generate a clinical database of common mitochondrial disease mutations in the Maltese population.

The Malta NGS Project: The Application of High Throughput Sequencing for the Identification of Mutations in Rare and Common Diseases

Ritienne Attard[1], Graziella Camilleri[1], Francesca Borg Carbott[1], Julia Camilleri[1], Adrian Pleven[1], Joshua Vella Harmsworth[1], Tiziana Felice[1], Philip Dingli[1], Karen Cassar[2], Rosienne Farrugia[1], Stephanie Bezzina Wettinger[1]

[1]Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta [2]Faculty of Medicine Surgery, University of Malta, Msida, Malta

The Malta NGS project (Harnessing and Maximising the Potential of Next Generation Sequencing) was designed to take advantage of the genetic history and composition of the Maltese population. The goal of the Malta NGS project is to utilise selected gene panels and exome sequencing to identify novel disease causing genes and mutations within a subset of diseases present in the Maltese, concomitantly setting-up high throughput sequencing (HTS) expertise in Malta. This project, funded by a national R&I grant (2012 programme), is a collaborative effort between scientists and clinical experts, thus employing the expertise of specialist clinicians in the identification and phenotyping of patients and the technical expertise of scientists in setting up the collections and carrying out laboratory analyses. Twenty conditions including both rare and common diseases (such as neuronal and metabolic conditions and disorders of the kidney, heart , eye and bone) are being investigated as part of this project. Additionally, the NGS project is serving to train a number of MSc and PhD students in this field. The vast amount of data which is being generated will require extensive bioinformatics analysis within the context of the specific disease, lifestyle and environmental influences which are also being captured during patient recruitment.

Parkinson Disease (PD) was one of the first conditions to be tackled as part of the Malta NGS project. Utilising samples from The Geoparkinson Study collection, a DNA collection from 200 patients with PD or Parkinsonism and 400 population controls, HTS was used to generate exon sequence data of a number of known candidate PD genes in a subset of samples from individuals with a family history or early disease onset (3rd decade of life). Two novel mutations were identified, one within *LRRK2* and one within *PINK1*. The p.N618S mutation in *LRRK2* appears to be geographically restricted to the local population where it is relatively common and appears to double the risk for PD.

The Maltese Acute Myocardial Infarction Study

Ritienne Attard[1], Julia Camilleri[1], Francesca Borg Carbott[1], Philip Dingli[1], Karen Cassar[2], Carine Doggen[3] Rosienne Farrugia[1], Stephanie Bezzina Wettinger[1].

[1]Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta.
[2]Faculty of Medicine and Surgery, University of Malta, Msida, Malta.
[3]Health Technology and Services Research, MIRA, University of Twente, Enschede, The Netherlands.

Cardiovascular disease is one of the major causes of death in developed countries. Malta has the highest death rate (men: 151.7 deaths/100,000; and women: 89.5 deaths/100,000) attributed to ischemic heart disease among its neighbouring countries. Myocardial infarction (MI) is influenced by a number of genetic, environmental and lifestyle factors. The Maltese Acute Myocardial Infarction (MAMI) Study was designed to explore these factors. It is a collaboration between the University of Malta and the Maltese Department of Health and it includes 423 cases with a first myocardial infarction as defined by the Joint European Society of Cardiology/American College of Cardiology committee, 465 population controls and 210 relatives of cases. Cases participated twice in the study, once at time of hospital admission and once at least six months after. Morning fasting blood samples were collected, processed and banked within an hour following standard protocols giving particular attention to control and recording of preanalytical variables. Extensive data was collected from an interviewer-led questionnaire, a review of medical history, use of medications, measurements of physical parameters including weight, height, blood pressure, waist and hip circumference, several biochemical, immunological, haematological and coagulation tests, RNA analyses of inflammation and Toll-like receptor related genes, several protein measurements as well as genetic analyses of genes with a focus on inflammation, coagulation and lipid metabolism. The impact of the conventional risk factors on MI was assessed. Levels of inflammatory molecules were elevated in cases compared to controls. Several haematological parameters including white blood cell counts, lymphocyte and monocyte counts, and inflammation-related mRNA transcripts, including NFKB and IL-1B, were elevated even in relatives of cases compared with controls having no family history of MI. The effect of some genotypes on the risk of MI was observed to be modulated by a number of factors including lifestyle and metabolic conditions. Next generation sequencing analysis using a candidate gene and biological pathway approach is being conducted. The study was funded through the Maltese R&I Programme 2008 and several MGSS and STEPS scholarships.

Variants Effecting Inflammatory Expression Profiles Identified by a Novel Biological Pathway Approach.

Attard Ritienne[1], Cassar Karen[2], Farrugia Rosienne[1], Bezzina Wettinger Stephanie[1]

[1]Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta
[2]Faculty of Medicine and Surgery, University of Malta, Msida, Malta

Introduction: The genes involved in the generation of an inflammatory immune response are many, with different combinations of variants possibly having a different effect. Approaches used to date have not yet unveiled the variants responsible for the generation of different inflammatory expression profiles.
Methods: In this family-based candidate gene approach, families were selected from the extreme ends of the phenotype distribution of inflammatory expression profiles, as assessed by multiplex ligation-dependent probe amplification assays on whole blood. Exome sequencing of inflammation-related, TLR and TLR-related genes was carried out on an Illumina HiSeq4000. SNPs and indels were compared between families with low and high expression profiles. Variants in genes coding for proteins which physically interact, carry out similar or overlapping functions and which participate in the same biological pathway were analysed per family.
Results: Patterns of TLR and inflammatory expression profiles tended to be similar in members of the same family. First-degree relatives share a considerable number of variants in inflammation-related genes, while very few differences were observed between families with low and high inflammatory expression profiles. While an accumulation of variants were identified in some TLRs, IRAK and MAPK genes, fewer or no variants were observed in molecules having a more critical role in the TLR signaling pathway including MyD88, and transcription factors.
Conclusions: The similar pattern of inflammatory expression profiles in families is possibly due to the presence of inherited factors as members of a family share a considerable number of variants in inflammation-related genes. Genetic variation influences the fine-tuning of the inflammatory response where the overall effect on a pathway is an accumulation of the effects of the variants within that pathway.

A Fruitful Fly Forward in Drug Screening for Neurodegenerative Diseases.

Michelle Briffa, Neville Formosa, Ruben Cauchi.

Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta

Neurodegenerative diseases, such as Alzheimer's disease (AD), are increasing in prevalence and the need for novel disease-modifying therapies is critical. Identifying compounds that modify disease progression has been a struggle - mainly due to the insufficient knowledge regarding the underlying pathophysiological mechanisms of these diseases. *Drosophila* models of neurodegenerative disease can be effectively exploited in drug screens for the identification of compounds and target disease mechanisms. Extracts derived from Mediterranean plants, were tested on *Drosophila* models of AD and found to be effective at an optimum concentration. Their effect was seen as a significant increase in the lifespan and at 50% survival rate, together with a significant improvement in the climbing ability of these AD models. The conclusions being drawn from this study present an opportunity to further investigate the medicinal effects of plant and herbal extracts on AD and other neurodegenerative models. Further studies may guide the development of these novel therapeutic drugs that modify or prevent disease progression with minimum side effects for patients.

The Genetics of osteoporosis and fragility fractures in Malta

Melissa Formosa, Angela A. Xuereb

Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta

Osteoporosis is a hereditary multifactorial skeletal disease characterized by low bone mass and strength leading to an increased fracture risk. Clinical risk factors (CRF), with or without BMD, are used to predict fracture risk. A number of gene variants have been found associated with low BMD and fracture susceptibility. However, fractures can occur independent of BMD. In an ongoing Maltese study, the candidate gene approach and linkage analysis coupled with next-generation sequencing approach were used to identify genes that might be responsible for BMD and fractures in Malta. A case-control collection of more than 1000 Maltese postmenopausal women was recruited and BMD measurements were performed. Women who suffered low-trauma fractures were classified as cases whereas women without a fracture history were included as controls. CRF including anthropometric and biochemical parameters were determined, and gene variants within the major bone pathways were genotyped. Indeed, a number CRF and gene variants were found associated with low BMD and/or increased fracture risk, some of which directly contributed towards an increased fracture susceptibility independent of BMD. Two Maltese families having three or more affected members with osteoporosis and/or a fracture history were genotyped using a genome-wide single nucleotide polymorphism linkage scan followed by whole exome sequencing (WES). This narrowed down the extensive list of genes within the linkage regions to a small number of variants within a few biologically plausible genes. The most potentially deleterious gene variants according to *in silico* tools were selected and functionality was confirmed using *in vitro* assays. In addition, the same genes are currently being studied using animal models to determine their possible involvement in bone developmental processes.

Rare Kidney Diseases in Children

Esther Zammit,[1,2] Valerie Said Conti,[3,4] Alex E. Felice[1,2,5]

[1]Department of Physiology & Biochemistry, Faculty of Medicine & Surgery, University of Malta; [2]The Malta BioBank, Centre for Molecular Medicine & BioBanking, University of Malta; [3]Department of Child and Adolescent Health, Mater Dei Hospital; [4]Department of Paediatrics, Faculty of Medicine & Surgery, University of Malta; [5]Department of Pathology, Mater Dei Hospital.

In developed countries, renal-replacement therapy has greatly reduced the likelihood that a patient with a rare kidney disease dies from disease progression. However, the quality of life of these patients who are often children, is often poor. The current clinical management of rare diseases is then characterised by limited knowledge of the underlying mechanism and natural history of the disease. A better understanding of the cause and pathophysiology of rare kidney diseases could enable early identification and treatment of patients who are at risk of progressing to kidney failure. Exploiting the recent advances in *-omics* technologies, the first two projects of the LifeCycle Kidney Research Programme will be presented. These research projects look at rare kidney diseases in children namely, congenital anomalies of the kidney and urinary tract (CAKUT), and congenital nephrotic syndrome of the Finnish-type.

Funding: LifeCycle Malta Foundation through the University of Malta Research, Innovation & Development Trust (RIDT), ENDEAVOUR Scholarships Scheme. The scholarship may be part-financed by the European Union - European Social Fund.

The quest of computer scientists to discover new drugs

Jean-Paul Ebejer

Centre for Molecular Medicine and Biobanking, University of Malta.

Computer-Aided Drug Design (CADD) plays an increasingly critical role in the drug-discovery process. CADD involves the application of computer algorithms to improve pharmaceutical productivity. These include algorithms for the identification of the biological target involved in a disease, binding-site detection, toxicity and side-effect prediction, and searching a database for molecules which exhibit a therapeutic effect against a particular protein of interest. The latter is known as Virtual Screening. The exponential increase in size of bioinformatics databases, such as the Protein Data Bank, helps to improve the accuracy of these computational methods. During this networking event, we will present current methods in this field and give an overview of possible overlaps with ongoing research at the University of Malta.

Study of the control of Human Haemoglobin gene expression using a unique Haemoglobin variant

Dr Alexander Camilleri, Alex E. Felice

Mater Dei Hospital, Ministry for Energy and Health (Health) and Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta

Haemoglobin research has been a core component of the Malta Biobank and haemoglobin diagnostic tests were performed for over 25 years. New mutations were found to be involved in the control of globin gene expression and could be used for the development of new therapies for haemoglobin diseases such as thalassemia and sickle cell disease. My research is focused on a unique Haemoglobin variant found in the Maltese population; Hb Foetal Malta I. This biomarker is quantified and mutations which may affect its expression will be searched. Data and samples from cord blood of children born in the last 10 years will be used.

A multi-omic approach to understand developmental globin gene switching

Laura Grech[1,2], Joseph Borg, Alex E. Felice[1,2,3]

[1]Department of Physiology & Biochemistry, Faculty of Medicine & Surgery, University of Malta; [2]The Malta BioBank, Centre for Molecular Medicine & BioBanking, University of Malta; [3]Department of Pathology, Mater Dei Hospital.

KLF1 previously known as eKLF is now considered a 'Master Regulator' of erythropoiesis. *KLF1* mediates the developmental switch from expression of foetal γ globin to adult β globin. A range of unrelated human erythrocyte disorders have now been assigned to variants in *KLF1,* and over 65 molecular variants have been recorded to date. Their haematological phenotypes range from the clinically silent In(Lu) type of Lu(a-b-) blood group, variability in the HbA_2 levels, congenital dyserythropoietic anaemia (CDA) and in most extreme cases hydrops foetalis secondary to profound anaemia. In 2010 sequencing revealed a nonsense mutation in the *KLF1* gene in a large Maltese family with hereditary persistence of foetal haemoglobin (HPFH). The HbF levels of the family members heterozygotes for the p.K288X mutation varied from 3.3% to 19.5%. This mutation was found to ablate the DNA binding domain of the key erythroid transcriptional regulator. Functional assays showed that KLF1 has a dual role in the regulation of foetal-to-adult globin gene switching. It acts directly on the *HBB* locus as a preferential activator of the *HBB* gene and secondly it acts indirectly by activating the expression of BCL11A which in turns represses the *HBG1/HBG2* genes. We have identified 3 other families with the p.K288X mutation and variable HbF expression. The KLF1 mutation on its own does not fully explain the difference in HbF levels between individuals heterozygotes for the p.K288X mutation. The aim of this reseach is to uncover the network of genes or gene products that interact with KLF1 in developmental globin gene control. Whole genome sequencing (WGS) and RNA sequencing was carried out on selected family members from FamFI and FamF2 and currently being analysed.