

MEETING REPORT

The European Iron Club, Annual Meeting at Guy's Campus, King's College London

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Both Clinical and Molecular Aspects of iron metabolism, its disorders and its regulation were discussed during the two day annual meeting of the European Iron Club held at the Guy's Campus, of King's College in London. Iron deficiency anaemia, and iron overload (whether due to sickle cell disease, sideroblastic anaemia, or genetic haemochromatosis) as well as the key role of the recently discovered antimicrobial peptide, hepcidin in the regulation of iron homeostasis and metabolism, the use of alternatives to animal models for studies on iron metabolism and the key role of iron in bacterial pathogen infections were presented in plenary presentations.

The European Iron Club is a well loved and venerable organisation which regroups professionals in biomedical inorganic iron research, and this year it held its annual meeting at the King's College London from the 13th to the 15th of September, 2007, organized by **Rob Evans** and **Bob Hider**. As usual, it served as an interface between clinicians and fundamental bioscientists, with a heavy emphasis on the contributions of the latter to the understanding of the former, with some 170 participants enjoying an Indian summer in the attractive surroundings of the Guy's Campus of King's College. Substantial support from a number of sponsors allowed the participation some thirty students through bursaries.

Sue Fairweather-Tait summarised the role of dietary factors in the aetiology and prevention of iron deficiency anaemia, which has important functional consequences. Iron absorption from many diets among vulnerable groups of the population (notably pregnant women and young children), particularly in developing countries, is insufficient to prevent iron deficiency. In these situations, diets contain factors such as phytates and phosphates which bind iron and prevent it being absorbed, and transition metal ions like calcium, which compete for iron absorption. There is good reason to believe that humans are not well adapted to modern diets, and that Paleolithic diets may well have been more effective in supplying dietary iron. Strategies currently being used to prevent iron deficiency include biofortification (notably by selective breeding to maximise plant ferritin), improving bioavailability by enhancers such as EDTA, and food fortification.

The magnetic susceptibility of iron supplements and their importance in the understanding of clinical magnetism-based diagnostic techniques were analysed by **F.J. Lazaro**. Studies by **Yahya Pasdar** showed that, compared with wild type, heterozygote carriers of the HFE (haemochromatosis) gene had lower rates of iron deficiency and iron

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deficient anaemia. Previous studies which showed a link between NdrG-1, a growth inhibitor and metastasis suppressor gene and iron metabolism were extended, and the presentation by **Marie-Bérengère Troadec** underlined the importance to take into account the effect of iron loading on NdrG-1, favouring the development of liver cancer in humans. The importance of iron status in the risk of developing hepatocellular carcinoma were further underlined by the upregulation of p53 (**Luca Valenti**).

Sickle-cell disease, first described by James Herriott in a young student in 1911, is characterised by chronic haemolytic anaemia, punctuated by sickling crises triggered by the “sickled” red blood cells. As **Swee Ley Thein** pointed out, due to recent population movements, it has become a global health issue, and is currently the most common, and fastest growing serious genetic disorder in many countries, including the UK. The molecular origin is the polymerisation of deoxy haemoglobin S (in which glutamic acid 6 in the beta chain is mutated to valine), leading to haemolysis, anaemia and vaso-occlusion. Long-term care programmes, with regular screening together with a range of preventative and pain prevention measures have a significant influence on patient prognosis, and many SCD patients now survive into their fifth decade. Transfusion therapy which aim to maintain HbS levels at 20-30%, are particularly successful in preventing recurrent stroke in young children. Current studies in collaboration with **Bob Hider** seek to establish whether hepcidin levels could be a useful index for predicting particular complications.

The adherence of granulocytes to endothelial tissue is a key step in the clearance of invading pathogens, and it was found (**Niki Georgiou**) that pharmacological forms of non-transferrin bound iron could modulate the degree of granulocyte recruitment in inflammation. It was observed that in C282Y HFE haemochromatosis gene expression of hepcidin was down-regulated, while calreticulin mRNA levels increased (**J.P. Pinto**), correlated with protection from oxidative stress, and suggesting a role for calreticulin as a modifier of the clinical expression of hereditary haemochromatosis. Hepatitis C virus (HCV) chronic hepatitis predisposes to iron overload which influences the prognosis negatively. In a study of 143 previously untreated Italian patients (**Luca Valenti**) it was found that genes involved in iron metabolism influenced iron overload and steatosis, but that the major burden was related to the HCV itself.

Sideroblastic anaemia designates a wide spectrum of disorders, all of which are diagnosed by the presence of pathologic iron deposits in erythroblast mitochondria, and were described in greater detail by **Alison May**. Mutations in a number of mitochondrial genes include the classic X-linked mutations in ALA-synthase, the first enzyme of the haem biosynthetic pathway, where the mutations have been mapped on the three-dimensional structure of the protein, but also in an ATP binding cassette protein involved in iron transport, glutaredoxin, a protein involved in F-iron-cluster export from mitochondria, in a thiamine transporter, in pseudouridine synthase 1 and deletions in mitochondrial DNA (Pearson's syndrome). The consequences of some of these mutations can be relatively easily understood in terms of our growing understanding of mitochondrial iron metabolism. However, the identification of other key players in cellular iron metabolism will allow the clinician to help families affected by some of the rare inherited conditions resulting in sideroblastic anaemias.

The potential physiological role of the recently discovered mitochondrial ferritin was discussed by **Sonia Levi**, who concluded that it was more likely to be involved in iron detoxification and protection against oxidative damage than in iron storage. In Erythropoietic protoporphyria, deficiency of erythrocyte ferrochelatase, the terminal enzyme of the haem biosynthetic pathway, results in accumulation of protoporphyrin, causing life-long acute photosensitivity. **Mark Worwood** reported a study of 178 cases,

all of whom had decreased erythropoiesis, which was matched by reduced iron absorption and supply. No correlation was found between erythrocyte protoporphyrin and other markers of iron status. Friedreich's ataxia, the most prevalent cerebral ataxia in children and adults is characterised by deficiency of mitochondrial frataxin, a protein now known to be important in FeS assembly, and massive mitochondrial iron accumulation. **Or Kakhlon** described how frataxin deficiency led to oxidative damage and mitochondrial membrane depolarisation. However, only the latter was followed by cell death, indicating that the use of iron chelators, such as deferiprone, which could redistribute iron between intracellular compartments, could repair damage caused by frataxin deficiency. This hypothesis was further developed in studies using fluorescent metalosensors to suggest that this chelator could move iron out of iron-loaded cell organelles to other cellular compartments or to the extracellular medium, and from iron-loaded macrophages to pre-erythroid cells. **Mayka Sanchez** presented studies on previously unidentified mRNAs which might have iron regulatory elements (IREs) in their untranslated regions which might interact with the well known iron regulatory proteins (IRPs). Several have been identified and are in the course of characterisation.

In view of the accumulating evidence that redox metal ions, like iron, contribute to the oxidative stress involved in the genesis of neurodegenerative diseases³, the potential use of iron chelators for the treatment of neurodegeneration was reviewed by **Bob Hider**. He described the work of his group to try to develop orally active iron chelators, capable of crossing the blood-brain barrier, but which do not inhibit key iron-containing enzymes.

In conditions of serious iron overload, the transport capacity of serotransferrin is exceeded and a highly toxic form of serum iron, non-transferrin bound iron is found in the circulation and rapidly accumulated by the liver. In another presentation from the group of **Ioav Cabantchik** it was shown that this form of iron could be rapidly transferred to the mitochondria of cardiomyocytes. **Emanuela Tolosano** showed that mice in which the gene for the haem-binding plasma glycoprotein hemopexin had been ablated accumulated iron in brain basal ganglia, suggesting a role for hemopexin in iron-related neurodegenerative diseases. Using an elegant microdialysis technique in a rat model of Parkinson's disease, induced by 6-hydroxyl dopamine, **Roberta Ward** showed that pretreatment with the iron chelators desferrioxamine or deferasirox reduced both hydroxyl radical formation and iron accumulation, underlining both the potential for chelation therapy in neurodegenerative diseases and the usefulness of the microdialysis technique for screening new iron chelators.

The first day ended with a poster session (some 60 posters were presented during the meeting), followed by a concert in Guy's Hospital Chapel given by Emily Pringle (violin) and Marcus Andrews (piano).

In her overview, **Martina Muckenthaler** highlighted the importance of using Cre/LoxP technology to specifically ablate HFE expression in duodenal enterocytes, macrophages and hepatocytes in order to study the role of hepcidin in the iron overload observed in genetic haemochromatosis (HFE). Whereas it was found that intestinal Hfe is dispensable for the physiologic control of systemic iron homeostasis under steady state conditions, in contrast HFE was proposed to localise to hepatocytes, with two other

³ Crichton, R.R. and Ward, R.J. Metal-based Neurodegeneration from Molecular Mechanisms to Therapeutic Strategies, John Wiley and Sons, Chichester, England, 2006, pp.227.

proteins involved in hereditary haemochromatosis, transferrin receptor 2 and hemojuvelin, which also regulate hepcidin expression.

There has been considerable interest in the last few years on the role of hepcidin in the regulation of systemic iron homeostasis. Clearly, the accurate determination of hepcidin levels in serum and urine are important, and several contributions addressed this question (**Erwin Kemna, B.A.C. van Dijk** and **S. Bansal** for serum and **S. Bansal, N. Campostrini** and **Heinz Zoller** for urine). The consensus seems to be that there is still no gold standard, and indeed there may be the additional problem that the hepcidin may contain a metal ion, either copper (**Heinz Zoller**) or iron (**Sébastien Farnaud**).

The molecular mechanism of hepcidin, acting on reticuloendothelial macrophages and duodenal enterocytes to coordinate body iron homeostasis was reviewed by **Kaila Srail**, who concluded that the effects of hepcidin, notably on ferroportin expressing cells, are tissue specific. **Abdel-Majid Khatib** presented results demonstrating the role of Furin-like proprotein convertases in the generation of active hepcidin from its precursor, highlighting the control of hepcidin processing as a potential therapeutic target. These results were extended by **Laura Silvestri**, who showed that an endoplasmic reticulum-located furin, up-regulated by iron deficiency, was involved in the generation of soluble hepcidin. A coculture of mouse hepatocytes and rat liver epithelial cells was shown to permit sustained, high level hepcidin mRNA expression (**Nadia Fatih**), allowing the screening of modulators of hepcidin expression during inflammation.

An original and potentially powerful system for studying iron metabolism in the fruit fly, *Drosophila melanogaster*, was presented by **Fanis Missirlis**, which has the particularity of not having either HFE, hepcidin or ferroportin. This results in an increased role for ferritin in iron homeostasis. Both H and L transcripts of ferritin are coexpressed during embryogenesis and both subunits are essential for embryonic development, while overexpression of ferritin impaired the survival of iron-deprived flies. Using GFP-tagged holoferritin it was confirmed that *in vivo* iron-loaded ferritin molecules traffic through the Golgi organelle and are secreted into hemolymph.

Carole Peyssonnaud presented results confirming that the von Hippel-Lindau/hypoxia-inducible transcription factor (VHL/HIF) pathway is an essential link between iron homeostasis and hepcidin regulation *in vivo*. Through coordinate downregulation of hepcidin and upregulation of erythropoietin and ferroportin, the VHL-HIF pathway mobilizes iron to support erythrocyte production. There is an emerging body of evidence implicating iron in carcinogenesis and this was the object of the presentations by the group of **Keith Roberts** and **Chris Tselepis**. In colorectal cell lines in which the tumour repressor APC was mutated, overexpression of c-myc was shown to result in increased transferrin receptor 1 and dimetal transporter 1 leading to increased iron loading. The progression of oesophageal metaplasias to adenocarcinoma was shown to be associated with over-expression of iron transport proteins, resulting in increased intracellular iron load and the exacerbation of carcinogenesis. **Pavle Matak** showed that macrophages are an essential component of the pathways through which hypoxia and H₂O₂ negatively regulate hepcidin production, while **Gaetano Cairo** reported that hypoxia-inducible factor 1 (HIF-1) and NF-κB pathways were involved in the modulation of transferrin receptor transcription in inflammatory conditions in macrophages.

In his introduction to the final group of oral presentations **Andrew Gorringe** reviewed the different strategies utilized by bacterial pathogens to scavenge iron from their host. These include the secretion of powerful iron chelators (siderophores) accompanied by high affinity energy-dependent transport systems to internalize this iron, and, in the case of pathogenic *Neisseria*, the expression of outer membrane receptors for human transferrin, lactoferrin, haemoglobin and the siderophores produced by other bacteria, together with the periplasmic and inner membrane machinery required for iron uptake. Chronic infection results in iron redistribution in different mice strains infected with *Mycobacterium*, inducing anaemia, accompanied by induction of Lipocalin 2 (**Maria Salomé Gomes**), a monocyte and neutrophil derived peptide which exerts antimicrobial effects by binding bacterial siderophores. **Celia Conesa** described studies to identify regions in human ceruloplasmin which interact with lactoferrin, using an overlapping peptide library, while **Maria Podinovskaia** presented her studies on iron status in macrophages during *Mycobacterium* infection using novel fluorescent iron sensors to measure free iron within the endosomal/lysosomal system. In the final presentation, it was hypothesized by **Theurl Igor** that Lipocalin 2 might play a role in the pathophysiology of haemochromatosis by transporting iron across cell membranes.

The concluding remarks by **Jo Marx** presented an interesting contrast between subjects which had preoccupied European Iron Club meetings in the distant past with those of today, and allowed the audience to see a selection of photographs illustrating the evolution of some of the better known members of Club over the years.

The final evening concluded with a cruise on the river Thames, accompanied by the Conference Dinner and a chance to dance the night away (at least until the cruise ship docked below the London Eye).

Next year's meeting will be organized by **Felix Funk** and **Peter Geisser** on the shores of the Lake of Constance in Saint Gallen.