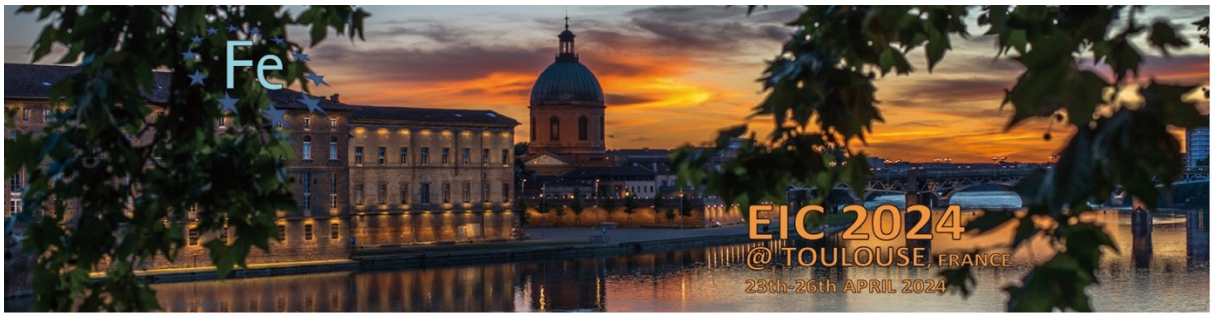

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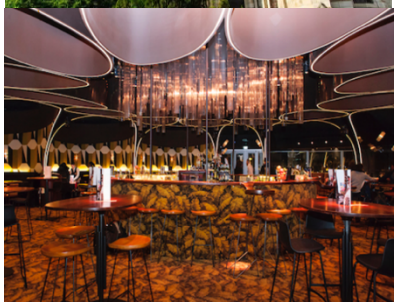

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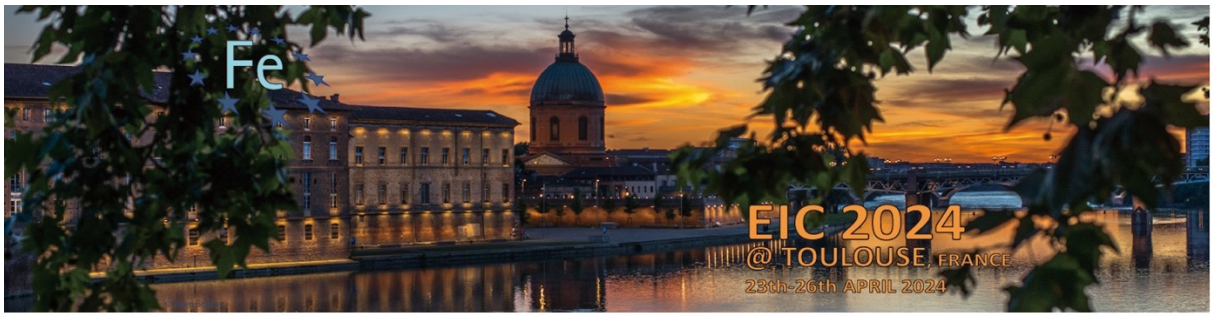
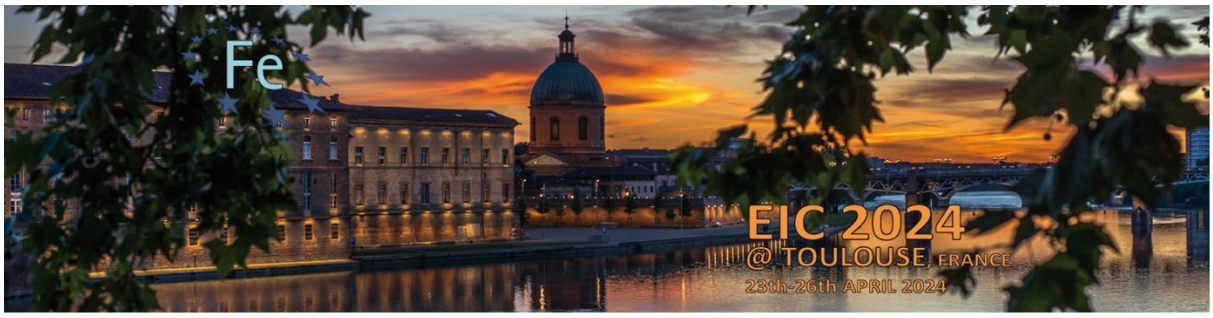


Contents



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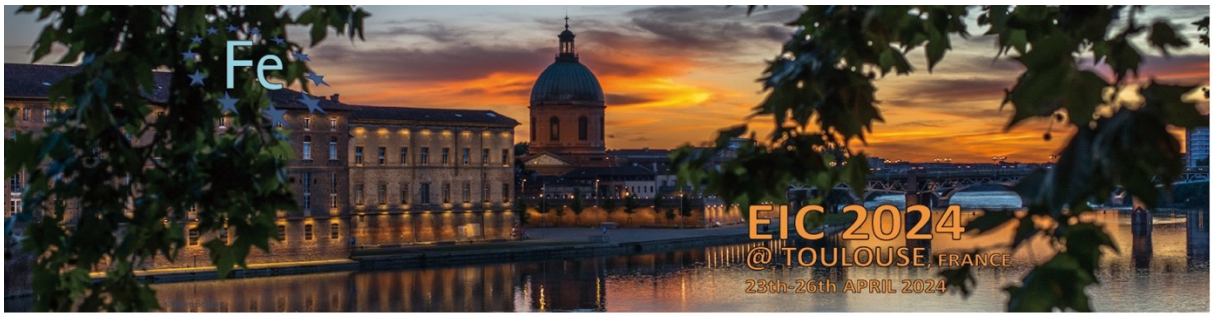

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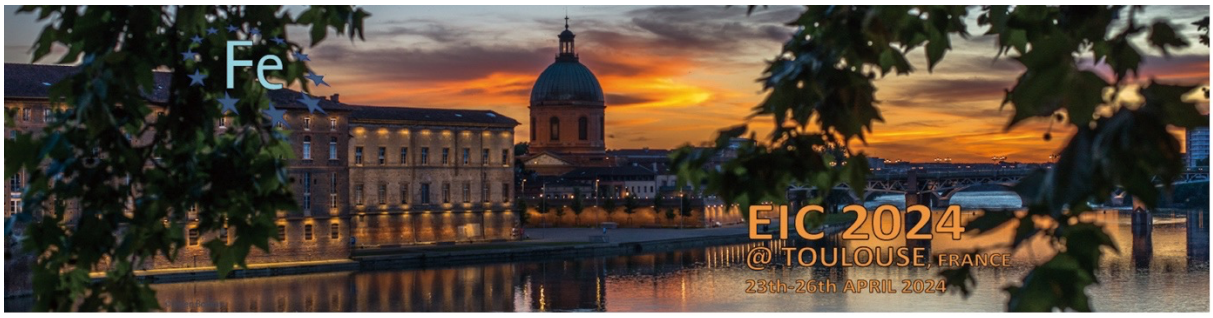

Oral Presentations session | OP-01 - Iron related disorders

ERYTHROFERRONE OVEREXPRESSION AMELIORATES ANEMIA AND ENHANCES KIDNEY FUNCTION IN A CKD MOUSE MODEL

CZAYA Brian [1], OLIVERA Joey [1], CASTRO Christian [1], JUNG Grace [1], NEMETH Elizabeta [1], GANZ Tomas [1]
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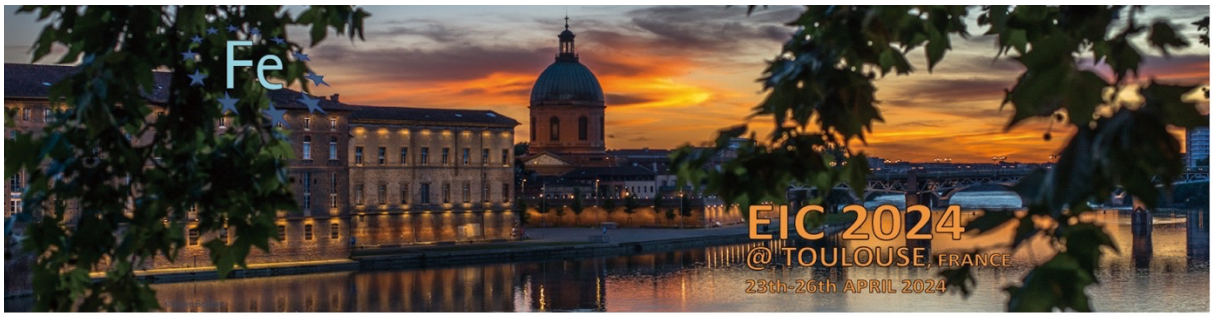
Chronic kidney disease (CKD) affects over 800 million people worldwide, most of whom develop anemia caused by relative erythropoietin deficiency, increased levels of the iron-regulatory hormone hepcidin, as well as absolute and functional iron deficiency. Erythropoietin-stimulating agents (ESAs) and iron supplementation ameliorate anemia in CKD but patients often become hyporesponsive to ESAs. Increasing ESA doses may heighten risks of thrombosis and cardiovascular mortality, highlighting the need for alternative strategies. Erythroferrone (ERFE) is an erythroid suppressor of hepcidin, facilitating iron mobilization for erythropoiesis. In CKD, diminished marrow erythroblasts contribute to decreased ERFE production, likely further increasing hepcidin.To explore the potential therapeutic use of ERFE in CKD, we tested the impact of augmented ERFE on systemic iron homeostasis and kidney function. Transgenic mice with erythroid overexpression of ERFE (ERFE-Tg) had similar iron stores to WT mice at weaning but became iron overloaded as they aged. To prevent early iron overload, we placed ERFE-Tg on 4ppm diet at weaning. 8-week-old male ERFE-Tg and wild-type (WT) mice were then fed 0.2% adenine-rich diet containing 100 ppm iron for 8 weeks to induce CKD. At 16 weeks, both WT and ERFE-Tg mice developed CKD and anemia but ERFE-Tg had higher hemoglobin, MCV and serum iron, as well as lower liver hepcidin mRNA/liver iron content ratio, indicating effective suppression of hepcidin by ERFE. Furthermore, kidney function (BUN and serum creatinine) was less impaired in ERFE-Tg. Thus, ERFE overexpression in adenine-induced nephropathy improves CKD-associated anemia and renal dysfunction, suggesting that ERFE has therapeutic potential in CKD.



Cell-autonomous osteoclast iron deficiency leads to osteopenia

CARVALHO OLIVEIRA Tiago [1], RAUNER Martina [2], MUCKENTHALER Martina U. [1], HOFBAUER Lorenz C. [2], ALTAMURA Sandro [1]

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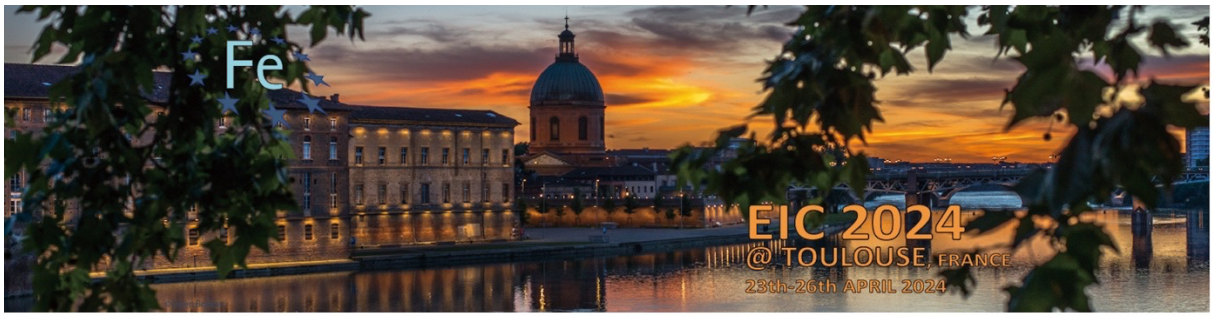
Maintaining optimal bone health requires a proper balance of iron levels. Conditions characterized by excessive iron, such as hereditary hemochromatosis, often lead to complications such as osteoporotic fragility fractures. However, it remains unclear whether impaired bone remodeling arises from direct variations in bone iron content or alterations in the iron status of other organs. In this study, we generated a mouse model with iron deficiency specifically in myeloid-derived cells, including osteoclasts ($FPN_{C326S}^{LysMCre+}$), due to the presence of the Fpn p.C326S gain of function mutation in the iron exporter Ferroportin (Fpn).

$FPN_{C326S}^{LysMCre+}$ mice maintain balanced hepatic and systemic iron levels despite a reduction in splenic iron content. Bone analysis revealed severe iron deficiency, with significant architectural change: a decrease in trabecular volume and thickness and an increase in trabecular space. Additionally, it was observed an increased number of osteoclasts per total bone perimeter, without alterations in bone formation rate per bone surface.

Ex-vivo osteoclast cell culture assays demonstrated that osteoclasts derived from FPN_{C326S} mice display increased bone resorption capacity without alterations in osteoclast formation. A similar phenotype was observed in wild-type osteoclasts treated with the iron chelator DFO, indicating that the phenotype may arise from intracellular iron deficiency.

In summary, our findings demonstrate that osteoclastic iron deficiency, even in the presence of balanced systemic iron levels, is sufficient to trigger significant alterations in bone microarchitecture due to increased osteoclast activity. This work holds promising translational implications, suggesting that modulating iron levels could serve as an adjunct to available antiresorptive therapies.



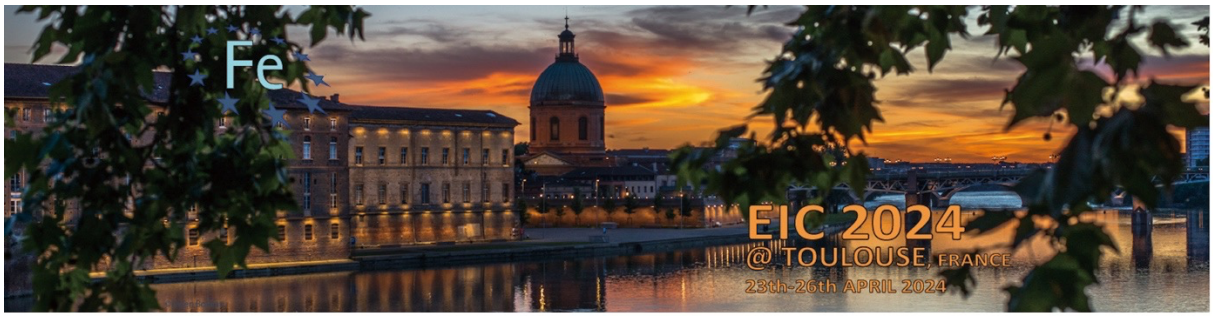
ENHANCED ERYTHROPOIESIS BY HEMATOPOIETIC TFR2 DELETION CORRECTS HYPERGLYCEMIA IN β-THALASSEMIA

DI MODICA Simona Maria [1], TANZI Emanuele [1], PETTINATO Mariateresa [1,3], PAGANI Alessia [1,3], SILVESTRI Laura [1,3], NAI Antonella [1,3]

[1] Regulation of Iron Metabolism Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy; [2] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [3] Vita-Salute San Raffaele University, Milan, Italy

Alterations of glucose metabolism and diabetes are common complications of β-thalassemia, mainly ascribed to organs dysfunction because of iron accumulation. However, β-thalassemia carriers, who do not experience iron overload, have a higher risk of developing glucose abnormalities compared to the general population. Thus, other factors likely contribute to metabolic dysregulation in β-thalassemia. We hypothesize that ineffective erythropoiesis (IE) might play a pivotal role. Indeed, hyperactive erythropoiesis in polycythemia mice results in systemic hypoglycemia.

Erythroid Transferrin Receptor 2 (TFR2) is a negative regulator of erythropoietin signalling. Indeed, Tfr2 deletion ameliorates anemia and IE in β-thalassemia models, likely increasing the metabolic activity of erythroid cells. Thus, hematopoietic *Tfr2* deletion, stimulating erythropoiesis, might promote glucose consumption, thus improving metabolic abnormalities in β-thalassemia. To address this point, we evaluated the metabolic profile of wt and thalassemic ($Hbb^{th3/+}$) mice with or without hematopoietic *Tfr2.* In line with patients' data, $Hbb^{th3/+}$ mice were hyperglycemic both in basal and in fasting conditions, without major changes in insulin levels. Interestingly, blood glycemia inversely correlated with the degree of anemia independently from iron overload in the different genotypes, with *Tfr2*-deficient mice showing reduced glycemia and improved response to a glucose tolerance test. Of note, transcriptomic analysis showed an increased expression of metabolic genes in *Tfr2*-deficient erythroblasts, mainly at the most terminal stages of maturation, indicating that glucose uptake and metabolism strongly support terminal erythroid differentiation. Overall, these results suggest that increased glucose consumption by the *Tfr2*-deficient hyperactive erythropoiesis reduces systemic glucose levels, thus correcting hyperglycemia in β-thalassemic mice.



COMBINING IRON RESTRICTION AND ERYTHROID MATURATION AGENTS HAS A SUPERIOR THERAPEUTIC EFFECT IN MDS

ANTYPIUK Ada [1], VANCE S.zebulon [1], SHARMA Richa [1], DÜRRENBERGER Franz [2], MANOLOVA Vania [2], **VINCHI Francesca [1]**

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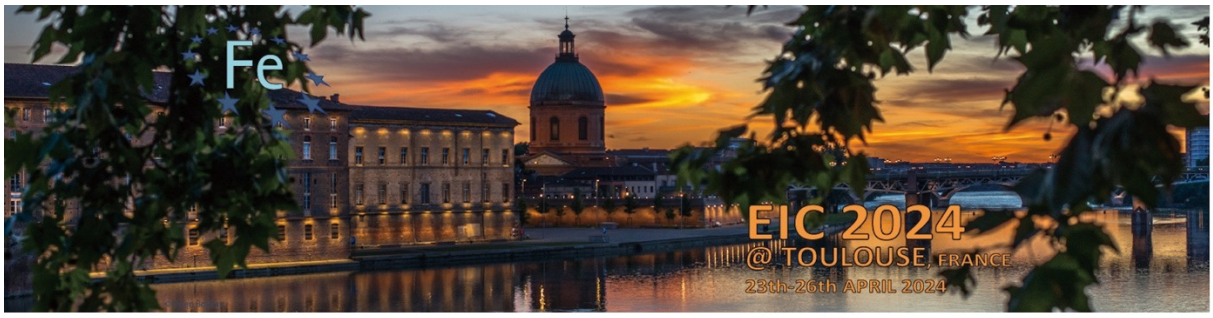
Patients with myelodysplastic syndromes (MDS) develop iron overload due to their underlying ineffective erythropoiesis and chronic transfusion therapy. The TGF-β superfamily ligands trap luspatercept, despite lacking disease-modifying activity, has become first-line treatment in low-risk MDS thanks to its ability to improve anemia by promoting EPO-independent maturation of late-stage erythroid cells. Here we investigated the effect of a therapy combining luspatercept with the FPN inhibitor vamifeport in the preclinical NUP98-HOXD13 MDS mouse model, with the hypothesis that drug combo could have additive benefits for the disease.

Vamifeport administration, either as single or combined treatment with luspatercept, corrected iron overload in MDS mice, by reducing serum iron, NTBI, and tissue iron loading. While both single agents ameliorated erythroid maturation and hematologic parameters, the combined therapy resulted in a further improvement of anemia and more effective erythropoiesis, as suggested by higher hemoglobin, hematocrit and RBCs, and decreased early erythroid precursors compared to single-drug treatments, effect likely resulting from improved progenitor survival and iron utilization. The HSPC pool and DNA damage were significantly ameliorated by vamifeport as single or combined treatment. Importantly, vamifeport, but not luspatercept, significantly attenuated myeloid expansion and myeloblasts in the MDS bone marrow, suggesting disease-modifying activity for this drug and revealing a major role of the iron status in myeloid skewing.

Overall, these data prove that combo therapies aimed at restricting iron and boosting erythroid maturation have superior effects in improving MDS pathophysiology, providing pre-clinical evidence for their application, and suggest they may offer more effective strategies for MDS treatment.

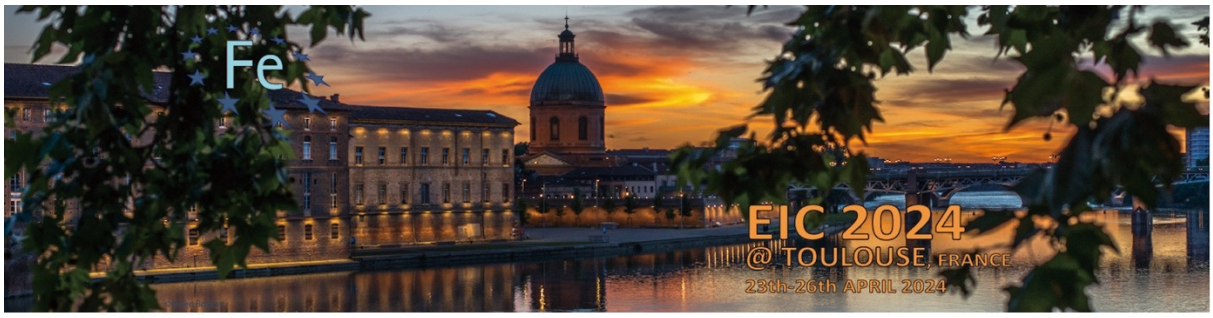


FERRIC CARBOXYMALTOSE INCREASES BONE FRACTURE RISK AND INHIBITS DMP1-MEDIATED αVβ3-INTEGRIN SIGNALIN

WAGNER Sonja [1], PAMMER Lorenz Michael [2], SARETTO Martina [1,2], PERTLER Elke [1,2], PANZER Marlene [1,2], SCHAEFER Benedikt [2], OBHOLZER Laura [2], FASERL Klaus [3], SARG Bettina [3], GLODNY Bernhard [4], TALASZ Heribert [3], WOLF Myles [5], TILG Herbert [2], ZOLLER Heinz [1,2]

[1] Christian Doppler Laboratory for Iron and Phosphate Biology, Medical University of Innsbruck, Innsbruck, Austria; [2] Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria; [3] Division of Medical Biochemistry, Protein Core Facility, Biocenter, Medical University of Innsbruck, Innsbruck, Austria; [4] Department of Neuroradiology, Medical University of Innsbruck, Innsbruck, Austria; [5] Division of Nephrology, Department of Medicine and Duke Clinical Research Institute, Duke University School of Medicine, Durham, United States

Objective: The intravenous iron formulation Ferric carboxymaltose (FCM) increases FGF23 and can cause hypophosphatemia. This study aims to identify the underlying mechanism and effects on bone. **Methods**: The translational study includes retrospective data in FCM- or Ferric Derisomaltose (FDI)-treated patients. The experimental studies compared FCM, FDI, and low-molecular weight iron dextran (LMWID). Iron deficient animals were treated with intravenous irons. MC3T3-E1 cells were used for osteogenic differentiation. Receptor binding studies were conducted with dentin-matrix protein-1 (DMP1) and αVβ3-integrin. Intracellular signaling was analyzed with phospho-proteomics. Surface charge and phosphate binding of the irons were compared. **Results**: In 289 intravenous iron-treated patients, FCM was associated with a significantly higher fracture risk than FDI, despite similar risks between groups prior to receiving intravenous iron. Bone marrow-free femora of FCM treated animals had significantly higher iron concentrations. Seven days post treatment, significantly more iron localized to the trabecular bone surface after FCM than after FDI or LMWID. Intact FGF23 plasma concentration was also significantly higher in FCM-treated animals. FCM had distinct phosphate-binding and pH-dependent charge properties. Only FCM inhibited DMP1 binding to αVβ3-integrin and MAP-Kinase signaling in cells. DMP1 is a known FGF23 inhibitor and important for bone turnover. Direct exposure to IV irons inhibited osteogenic differentiation, where intracellular iron was highest and collagen production was inhibited only after FCM. **Conclusion**: The charge and affinity of FCM to phosphate correlates with inhibition of DMP1 binding and enhanced localization to trabecular bone. FCM treatment affects bone beyond hypophosphatemia and intact FGF23 induction.



A genome-wide meta-analysis connects iron homeostasis to metabolic disease through poly-unsaturated

PRAGGASTIS Sophia [1], GILLY Arthur [2], CROWELL Beth [1], IVANOVA Larisa [1], ECONOMIDES Aris [1], HATSELL Sarah [1], BOVIJN Jonas [2], DE Tanima [2], **LOB Heinrich [1]**

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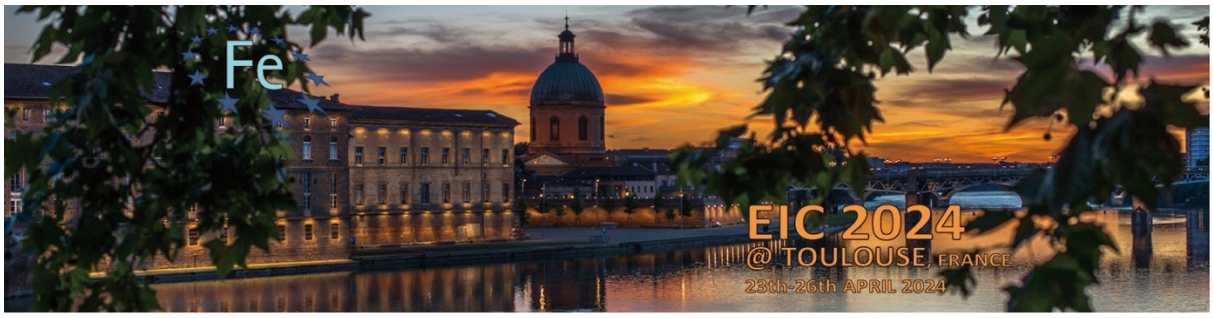
Individuals with genetic iron overload diseases have a higher incidence of metabolic diseases. In individuals without iron overload, serum levels of the iron biomarkers ferritin and transferrin positively correlate with type 2 diabetes. The molecular mechanisms connecting iron and metabolic dysfunction remain unknown. We performed a large-scale genome-wide association study (GWAS) of iron and glucose-related biomarkers (N = 480,389). We identified a variant, rs174560, in the FADS1/FADS2 locus associated with reduced iron and glucose levels. The variant is an expressed quantitative trait loci (eQTL) associated with reduced FADS1 expression. FADS1 plays a critical role in long chain polyunsaturated fatty acid (PUFA) synthesis. Hepatic depletion of FADS1 improves glucose clearance and limits adipose tissue expansion in vivo. Variants in the FADS1 locus are associated with blood glucose levels in previous GWAS, however, the mechanism underlying this association is unclear. Using CRISPR/Cas9 and siRNA, we targeted FADS1 expression in the liver of C57Bl/6 mice. Specific reduction of FADS1 impaired PUFA synthesis, reduced serum iron, and elevated Hamp expression confirming FADS1 involvement in iron homeostasis in vivo. In $HFE^{-/-}$ mice, a genetic model of iron overload, FADS1 reduction significantly normalized serum aspartate aminotransferase levels (AST). High circulating AST levels indicate liver damage and reducing FADS1 expression may be protective against liver damage during iron overload. Our work shows a strong connection between fatty-acid biology and trace metal homeostasis. The connection to glucose metabolism found in GWAS could be secondary to improved liver health, as liver function and glucose metabolism are tightly connected.



A NOVEL MODEL OF X-LINKED SIDEROBLASTIC ANEMIA (Alas2-KO, XLSA) WITH RING SIDEROBLAST, SEVERE ANEMIA

CASTRUCCIO CASTRACANI Carlo [1], GUERRA Amaliris [1], BREDA Laura [1], PAPP Tyler [2], RADAELLI Enrico [2], ASSENMACHER Charles-Antoine [2], FINESSO Giovanni [2], TAM Yink [4], MUI Barbara [4], FONTANA Simona [3], RIGANTI Chiara [3], FIORITO Veronica [3], PETRILLO Sara [3], TOLOSANO Emanuela [3], PARHIZ Hamideh [2], **RIVELLA Stefano [1,2]**

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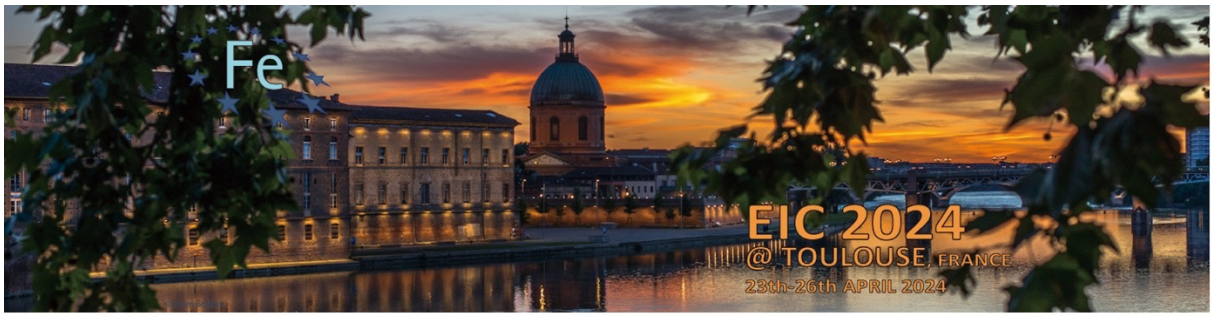
X-linked sideroblastic anemia (XLSA) is a congenital anemia caused by mutations in Alas2, a gene responsible for heme synthesis. Most patients carry hypomorphic mutations, with variable phenotypic manifestations. Treatment options include pyridoxine supplements, transfusions, and allogeneic bone marrow (BM) transplantation. To study an inducible severe manifestation of XLSA, we engineered a conditional Alas2-KO mouse model using two approaches: tamoxifen administration and treatment with lipid nanoparticles (LNP) carrying Cre-mRNA and conjugated to the anti-CD117 antibody. Alas2-KO-BM animals displayed fetal anemia characterized by ineffective erythropoiesis (IE) and ring sideroblasts, the first model showing this phenotype. Erythropoiesis in these animals showed expansion of polychromatic erythroid cells, decreased activity in the electron transport chain and mitochondria's function, and reduced activity of crucial Tricarboxylic Acid (TCA) cycle enzymes. The IE was associated with marked splenomegaly, high erythroferrone, low hepcidin levels, and iron accumulation in the BM, liver, and spleen. To investigate a gene therapy approach for XLSA, we developed a lentiviral vector (X-ALAS2-LV) that exploits a globin promoter and enhancers to direct human ALAS2 expression in erythroid cells. Infusion of BM cells with 0.6-1.4 copies of the X-ALAS2-LV in Alas2-KO-BM mice rescued these mice by improving erythropoiesis and tissue iron accumulation. These findings suggest our vector could be curative in XLAS patients. Since X-ALAS2-LV rescues Alas2-KO-BM mice, we are now modifying X-ALAS2-LV with ALAS2 human mutations to study milder forms of XLAS and develop alternative therapeutic interventions, such as pharmacological treatments and in vivo gene editing.



The role of cell extrinsic Nrf2 in hematopoietic stem cell transplantation with iron overload

REIS Joana [1,2], PEREIRA Carolina [1,2], LOPES Marta [1,2], TELES Maria José [1,3], MOSTEO LOPEZ Laura [1], PORTO Graça [1,4,5], L DUARTE Tiago [1], DUARTE Delfim [6,7,8]

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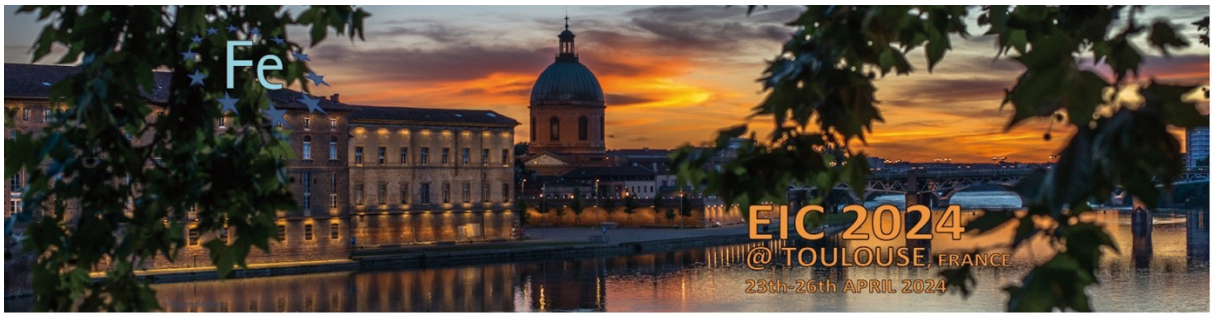
Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) often present iron overload (IOL). HSCT patients with high labile plasma iron show increased non-relapse mortality. NRF2 is a master anti-oxidant regulator, protecting against iron toxicity. The role of NRF2 in HSCT is not fully understood. We hypothesize that cell extrinsic NRF2 expression may impact HSCT outcome when IOL is present. To evaluate non-hematopoietic NRF2, we generated BM chimeras using WT, Nrf2-/-, Hamp1-/- and Nrf2-/-Hamp1-/- as recipients where WT BM cells were transplanted. We observed that, unlike single Hamp1-/- or Nrf2-/- recipient mice, Nrf2-/-Hamp1-/- recipients had shorter survival, indicating failure in HSC engraftment. Notably, treatment of Nrf2-/- mice with iron dextran (FeDx) prior to irradiation and transplant recapitulated the phenotype of double-KO mice. Since the liver systemically regulates hematopoiesis and considering that Nrf2 plays a protective role against iron toxicity in the liver, we repeated the experiment with Alb-CreT/+;Nrf2fl/fl recipient mice. The deletion of hepatic Nrf2 had no impact in the survival or success of BM transplant. We next hypothesized that niche cells might be involved. Sorted LepR+ MSCs from FeDx-treated mice showed increased expression of downstream Nrf2 target genes Nqo1, Gclc and Hmox1 while serum CXCL12 remained unaltered, suggesting that another cell type is involved, such as endothelial cells. These results suggest that Nrf2 is a player in HSCT but the critical cell type and molecular mechanism involved remain unknown. Our work may contribute to improve the success rate of HSCT where the use of Nrf2 agonists might be of interest.



The effect of altitude of residence on dietary iron absorption in iron depleted women of reproductiv

MORETTI Diego [1], LIRIA Reyna [3], SCHEUCHZER Pornpimol [1], BOURGOS Gabriela [4], ZEDER Christophe [2], STOFFEL Nicole [2,5], ZIMMERMANN Michael [5]

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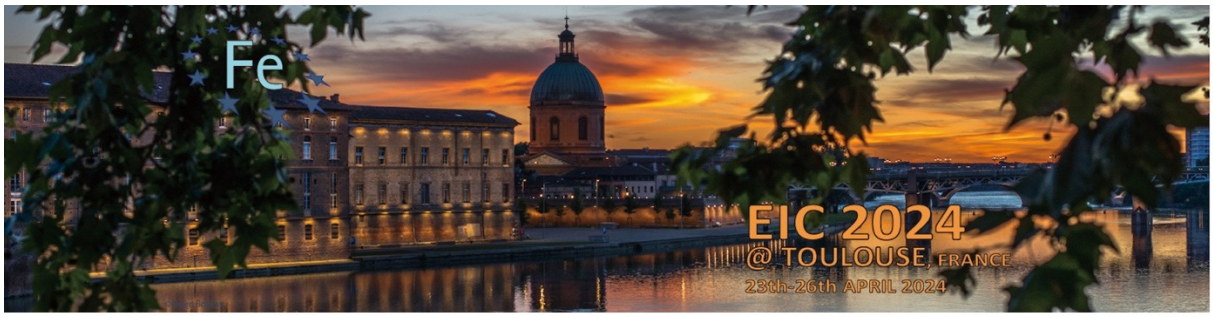
Background: Acute changes in altitude cause increase systemic iron clearance, erythropoiesis and iron absorption, but whether long-term high-altitude residents have higher iron requirements and iron absorption, has not been investigated in detail. Objectives: To assess and compare dietary iron absorption from iron biofortified potatoes in people living and Huancavelica (3670 m) and Lima (sea level) matched for iron status.

Methods: We aimed to recruit 40 generally health women of reproductive age in each study site with low plasma ferritin (PF<25 µg/L). Fasting subjects consumed identical, standardized 500g of potato based meals divided in breakfast and lunch labelled with 57FeSO4 on 5 consecutive days. Iron absorption was assessed by measuring the shift in iron isotopic composition in red blood cells 14 days after the last test meal administration.

Results: We recruited 40 subjects in each study site and 38 subjects completed the study. At high altitude, unadjusted Hemoglobin (15.0 vs 11.2 g/dl), EPO (16.6 vs 4.4 iU/L) and retinol binding protein (1.4 vs 1.2 µmol/L) were higher than in the group at sea level (P<0.05). There were no differences in PF, %TSat, Erythroferrone or inflammation but hepcidin tended to be higher at high-altitude (P=0.053). Fractional iron absorption from biofortified potatoes adjusted for iron status (PF=15 µg/L) was 9.1% at high altitude vs 6.8% at sea level (P<0.05). Conclusion: These data suggest one of the chronic adaptations to high altitude is an increase in fractional iron absorption from the diet, possibly to maintain the higher RBC mass needed at high altitude.



Gut-derived erythroferrone contributes to intestinal homeostasis

MEDJBEUR Thanina [1], DESQUESNES Aurore [1], MAS-OREA Xavier [1], ROY Maryline [2], DUMAY Anne [2], BARREAU Frédérick [1], KAUTZ Leon [1]

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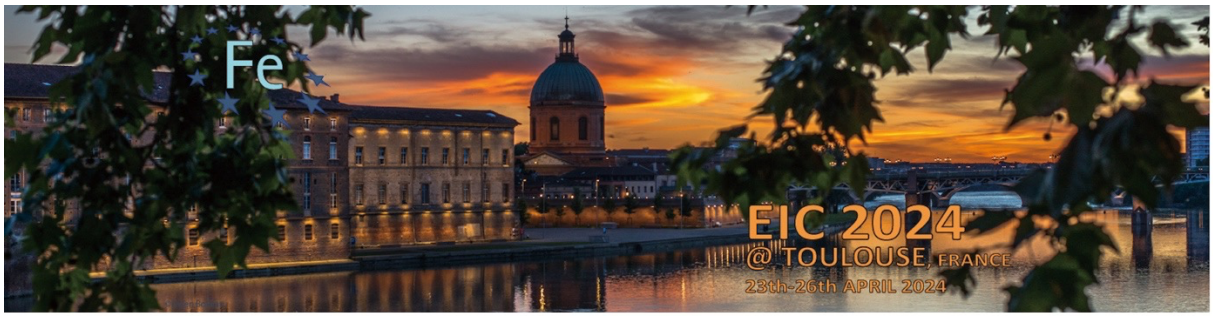
The hormone erythroferrone (ERFE) is secreted by erythroid progenitors in the marrow during stimulated erythropoiesis. ERFE represses hepcidin to ensure the supply of iron to the erythron by inhibiting the BMP/SMAD signaling. However, ERFE is also expressed in the gastrointestinal tract (Kautz, Nat Genet 2014), in which its function is unknown.

We explored the role of ERFE in intestinal homeostasis using wild-type (WT) and Erfe-deficient (Erfe-/-) mice. The mice were subjected to an experimental colitis induced by dextran sodium sulfate (DSS), a model of inflammatory bowel disease.

We first determined by in situ hybridization that Erfe mRNA was expressed in the colon in intestinal epithelial cells at the bottom of the crypts and in secretory cells. Interestingly, Erfe-/- mice exhibited shorter crypts and increased paracellular permeability in the ileum and the colon compared to WT mice. When challenged with DSS, Erfe-/- mice developed a more severe inflammatory phenotype than WT mice. Indeed, Erfe-/- mice presented with increased body weight loss, macroscopic (colitis, bleeding, colon length) and microscopic inflammatory scores (edema, mucus depletion, immune cells infiltration…) and premature death compared to WT mice. Consistent with the alteration of barrier function, Erfe-/- mice exhibited a blunted immune response with increased expression of inflammatory cytokines and antimicrobial peptides in the large intestine. Finally, using hematopoietic chimeras, we demonstrated that the protective effect was not conferred by hematopoietic ERFE. ERFE plays a role in maintaining intestinal homeostasis. We are currently investigating the mechanisms triggered by ERFE in the colon using mice and organoid models.



ASSESSING TRANSFERRIN RECEPTOR 2 FUNCTION IN INTESTINAL INFLAMMATION AND BONE LOSS DURING COLITIS

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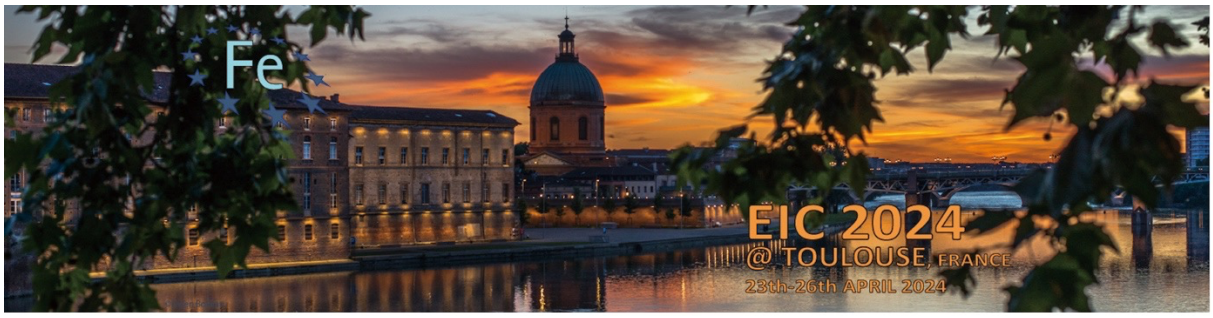
Osteopenia is a common complication of inflammatory bowel disease. Transferrin receptor 2 (Tfr2) is a major regulator of systemic iron levels, bone mass, and inflammation. Given iron's vital role in immunity and Tfr2's involvement in maintaining iron homeostasis, we hypothesize that Tfr2 deficiency impacts colonic inflammation progression and subsequent damage to bone tissue. Twelve-week-old male Tfr2-deficient mice (*Tfr2*$^{-/-}$) and wild-type (*Tfr2*$^{+/+}$) littermates as well as mice lacking Tfr2 in myeloid cells (*Tfr2*$^{fl/fl}$*;LysMCre+*) were analyzed for their susceptibility to experimental colitis. Colitis severity was assessed through changes in body weight, disease activity index (including stool consistency and bleeding), and colon length. Colon, serum, and bones were collected for qPCR, ELISA, flow cytometry, and µCT.
Colonic inflammation progressed more robustly in *Tfr2*$^{-/-}$ mice, characterized by significant colon shortening [1.5-, 1.2-fold; $p<0.05$], elevated colonic mRNA levels of *Tnfa*, *Il1b*, *Il6*, *Nos2*, *Rankl/Opg* [3-, 5-, 8-, 3.5, 2.5-fold; $p<0.05$], and increased infiltration of macrophages (F4/80^{+}) and monocytes (Ly6C^{+}) in the colon [2-, 2.2-fold; $p<0.01$] in comparison to *Tfr2*$^{+/+}$ mice. Furthermore, colitic *Tfr2*$^{-/-}$ mice exhibited enhanced systemic bone loss, higher serum levels of TRAcP5b, along with lower P1NP [-7.9%, 1.4-, 1.5-fold; $p<0.05$] compared to colitic *Tfr2*$^{+/+}$ mice. To assess iron burden contribution to colitis progression, we used *Tfr2*$^{fl/fl}$*;LysMCre+* mice with normal iron loading. These mice displayed increased disease development, substantial reduction in colon length, and increased bone loss [1.2-, 1.3-fold, -6.8%; $p<0.05$] compared to *Cre-* mice. Taken together, these findings underline a protective role of Tfr2 in the progression of colitis and colitis-induced bone loss.



IRON REGULATORY PROTEINS 1 AND 2 HAVE OPPOSING ROLES IN REGULATING INFLAMMATION

AZOULAY-BOTZER Bar [1], ZAYDEL Kristina [1], GHATPANDE Niraj [1], HARRER Aileen [2], GUTTMANN-RAVIV Noga [1], BHUSHAN Sudhanshu [2], MEINHARDT Andreas [2], **MEYRON-HOLTZ Esther [1]**

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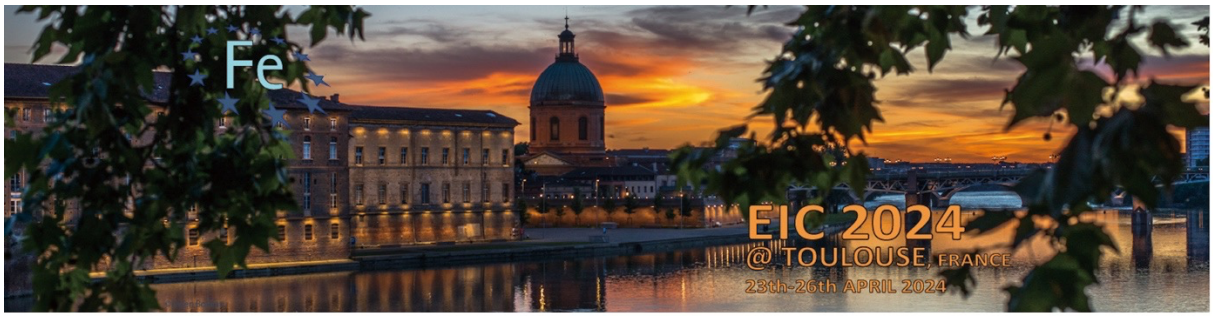
Iron is an essential nutrient, but excessive amounts can be toxic. Consequently, tight regulation of iron-homeostasis has evolved. Iron accumulation was demonstrated in inflamed tissue-lesions of patients with chronic inflammation, suggesting an inflammation-induced dysregulation of iron homeostasis. Based on this observation, we propose that impaired iron regulation influences the course of inflammation. To test this hypothesis, inflammatory response was studied in bone marrow-derived macrophages (BMDM) from mice with targeted deletions of the iron regulatory proteins (Irp)1 or Irp2, and in mouse models for rheumatoid arthritis (RA) as well as in uropathogenic Escherichia coli (UPEC) mediated epididymo-orchitis.

Irp1-/- BMDM showed a weaker, and Irp2-/- BMDM showed a stronger response to inflammation as demonstrated by the activation of the “mitogen-activated protein kinases” (MAPK) signaling pathways and inflammatory cytokine-production (Tnfα, Il-6). Following induction of inflammation, Seahorse analysis revealed differences in energy metabolism suggesting a more anti-inflammatory phenotype (M2) for Irp1-/- BMDM, and a more pro-inflammatory phenotype (M1) for Irp2-/- BMDM. Similarly, UPEC-mediated epididymo-orchitis elicited a significantly lower production of inflammatory cytokines (Tnfα, Il-6, Il1-β) and chemokines (Cxcl2, Ccl2) in the testis of Irp1-/- mice. In contrast, following infection, neutrophil-infiltration increased in the testis of Irp2-/- mice. Furthermore, deletion of Irp1, in a mouse model for Crohn’s disease and RA, completely abolished the intestinal inflammation, and reduced the inflammation in the joints.

Our data suggest opposing inflammatory responses of Irp1 and Irp2, and shed light on the intricate interplay between Irp1 and Irp2, cellular iron metabolism, energy metabolism, and macrophage polarization during inflammation.



Role of cutaneous hepcidin in graft-versus-host disease (GvHD)

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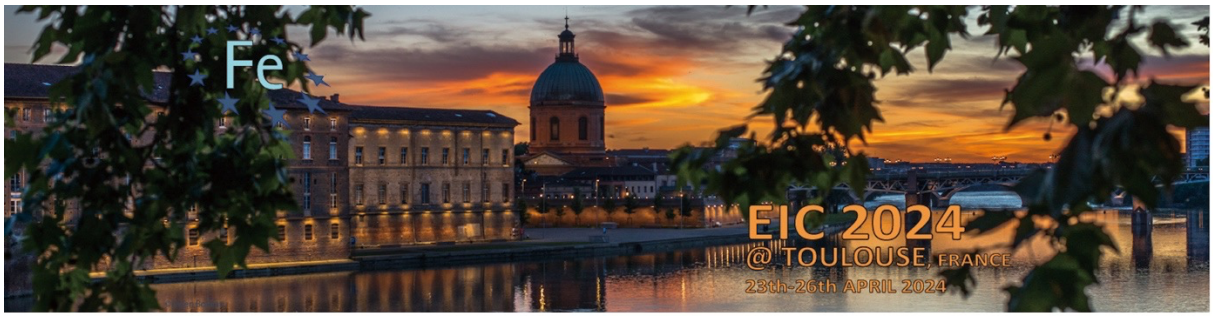
Acute graft-versus-host disease (GVHD) is a major cause of mortality in patients undergoing hematopoietic cell transplantation (HCT) for hematologic malignancies. Nearly 50% of allo-HCT patients will develop acute GVHD, characterized by an exacerbated inflammatory response and organ toxicity, the skin being the most commonly involved organ.

Hepcidin, the key regulatory hormone of iron homeostasis, is primarily produced by hepatocytes, but many cells and tissues express this hormone in pathological conditions. We have generated a new mouse model overexpressing hepcidin in keratinocytes and demonstrated a marked phenotype including, alopecia and epidermis thickening with local iron retention, together with a dramatic increase of IL-1β, TNF-α, CCL2, CXCL16 and CCL8 expression, and a massive immune cell infiltration, especially T lymphocytes, in the skin. Strikingly, this phenotype resembles some characteristics of patients suffering from GVHD, particularly the early events of the disease. Importantly, our data in skin biopsies of GVHD patients revealed for the first time an increase of hepcidin expression.

Interestingly, in control mice, performing only the irradiation conditioning regimen (prior to transplantation) is sufficient to induce skin hepcidin expression with concomitant iron accumulation, questioning the role of iron in the early steps of GVHD, in particular with regards to T cell activation. There is no doubt that studying the impact of iron and hepcidin in GVHD, both in murine models and in humans, may have potential for novel preventive/therapeutic drugs that are urgently needed to prevent/cure GVHD.



IRON ACCUMULATION AND METABOLIC CHANGES IN MONOCYTES CORRELATE WITH DISEASE SEVERITY IN SEPSIS

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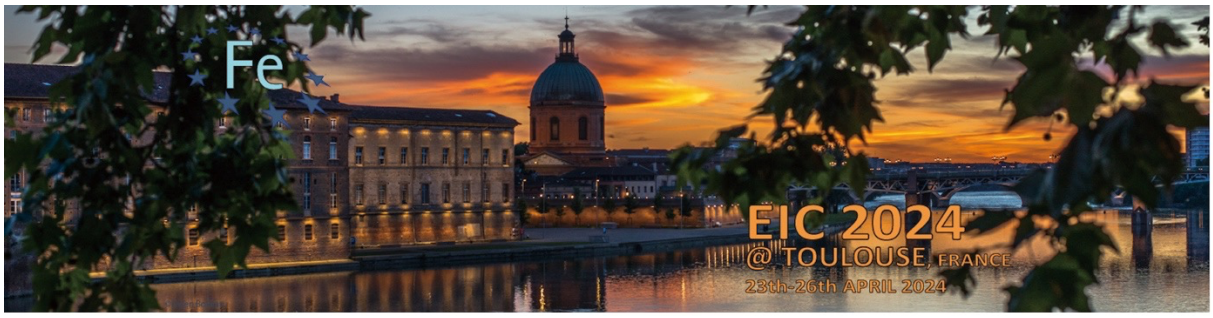
Sepsis is a frequent, often lethal syndrome caused by a maladaptive immune and metabolic response to infection. Alterations of iron metabolism play a crucial role in sepsis immunology. Iron markers not only indicate inflammation but reflect upon an active crosstalk with cellular immunity and can indicate prognosis and mortality of critically ill patients. Moreover, severe iron-deficiency at discharge from hospital is an independent predictor of poor physical recovery, making allogenic blood transfusions necessary. As of now, the mechanisms that link iron metabolism and sepsis progression are incompletely understood. We performed an observational study to analyze changes in iron parameters during the course of gut-origin sepsis. Whole blood was drawn daily from day 1 to day 5 after sepsis diagnosis and the patient's immunological status, systemic iron parameters and the iron content of CD14+ monocytes was quantified by atomic-absorption-spectrometry. We show that systemic iron parameters correlate with the severity of sepsis, in that serum iron and transferrin saturation were significantly lower in severely affected patients compared to those with a mild sepsis. Patient derived monocytes were severely iron-loaded during sepsis progression in a manner that correlated with disease severity and systemic inflammation. Consistently, the mRNA expression of transferrin receptor 1 was decreased. Seahorse analysis investigating the monocytic metabolic state provides indications that monocytic iron loading may alter mitochondrial function pointing towards a strong link between iron metabolism and monocyte function that needs to be elucidated further.



ROLE OF LIPOCALIN 2 AND IRON METABOLISM IN CANCER AND GLUCOCORTICOID-INDUCED SKELETAL MUSCLE ATROPHY

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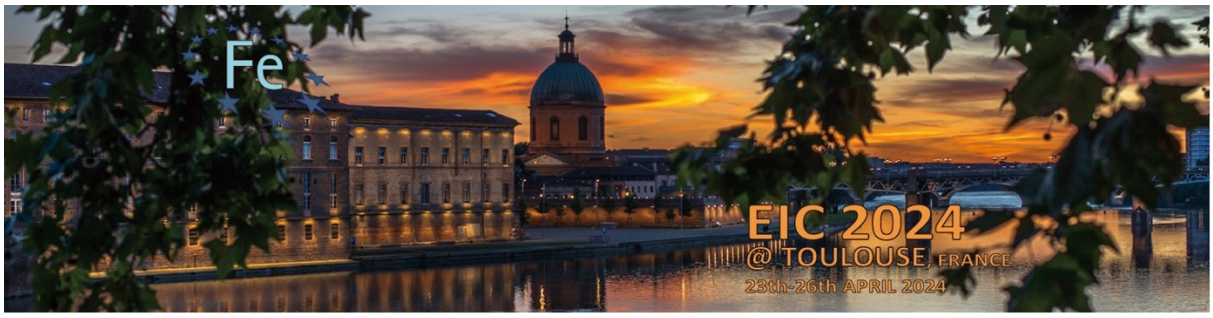
Cachexia is a wasting syndrome, marked by severe skeletal muscle atrophy, significantly increases mortality in various diseases including cancer, yet its mechanisms remain poorly understood. We previously identified major alterations of iron metabolism in the skeletal muscle of tumor-bearing mice and in particular a decrease in mitochondrial iron, resulting in mitochondrial dysfunction. However, the underlying causes of these alterations in muscle iron metabolism remain unknown. Lipocalin 2 (LCN2) an iron-sequestering protein, has been identified as one of the most upregulated genes in various models of skeletal muscle atrophy. Therefore, we investigated the role of LCN2 in skeletal muscle atrophy associated with cancer and chronic glucocorticoid treatment. LCN2 levels surged in muscles of both C26 tumor-bearing mice and dexamethasone treated mice. In vitro, LCN2 overexpression reduced C2C12 myotube diameter, while its silencing protected myotubes from dexamethasone-induced atrophy. In vivo, we found that aminoglutethimide, a drug inhibiting glucocorticoids synthesis, suppressed LCN2 expression in the skeletal muscle of C26 tumor-bearing mice thus suggesting that elevated levels of glucocorticoids are responsible for the upregulation of LCN2 in the C26 model Dexamethasone treatment, in vitro and in vivo, significantly reduced mitochondrial iron levels, mirroring C26 model iron metabolism alterations. Our finding shed light on cancer and glucocorticoid-induced skeletal muscle wasting mechanisms, highlighting LCN2 as a potential therapeutic target for muscle wasting diseases.



CONTRIBUTION OF HIF2A IN IRON BIOAVAILABILITY AGAINST ADHERENT INVASIVE ESCHERICHIA COLI INFECTION

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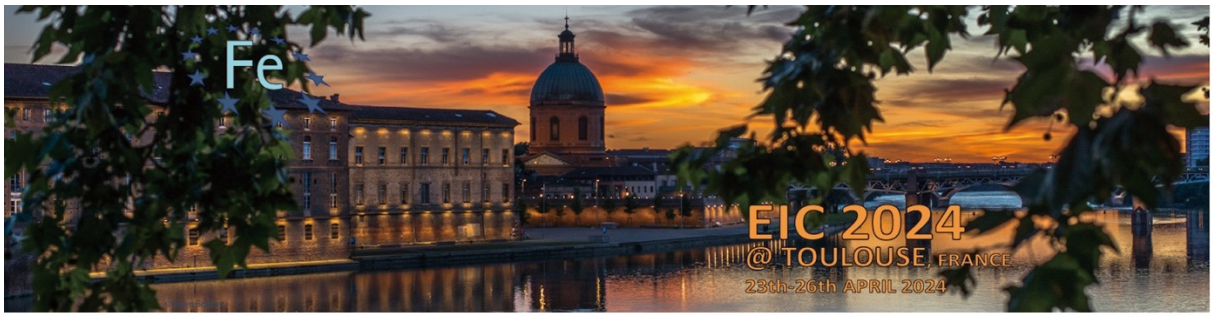
Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract. CD is strongly associated with expansion of the pathobiont adherent-invasive E.coli (AIEC) in the ileum, which plays an important role in CD pathogenesis. Comparative genomics revealed that AIEC genomes are enriched in iron acquisition genes relative to commensals. In line with this observation, iron has been described to be one of the key nutrients to favor AIEC virulence in vitro and our ongoing study demonstrated that AIEC infected-mice (LF82 strain) had a more persistent infection under iron supplementation.

To limit nutrient availability to pathogens, vertebrates have evolved diverse mechanisms, known as "nutritional immunity". Based on our preliminary results, we hypothesize that the transcriptional factor HIF-2α, known to regulate iron absorption, could be involved in the nutritional immunity against AIEC by restricting iron bioavailability in the gut. We infected mice lacking HIF-2α specifically in the intestinal epithelium (KOHIF2IEC) and WT littermates with LF82 and observed that, 4 days post-infection, KOHIF2IEC failed to restrict LF82 infection compared to WT mice. This persistent infection in KOHIF2IEC was associated with a defect in DMT1 expression (qPCR and WB) and a decrease of FTL expression (WB) in the ileum of infected KOHIF2IEC mice compared to WT. Analyses of a cohort of AIEC-associated CD patients is ongoing to correlate AIEC infection and HIF-2α target gene expression in ileum.

These results, showing that HIF-2α is critical to limit AIEC colonization, suggest that targeting intestinal HIF-2α can serve as a potential therapeutic for AIEC-associated CD.



IRON STATUS AND THE RISK OF SEVERE INFECTION IN AFRICAN CHILDREN

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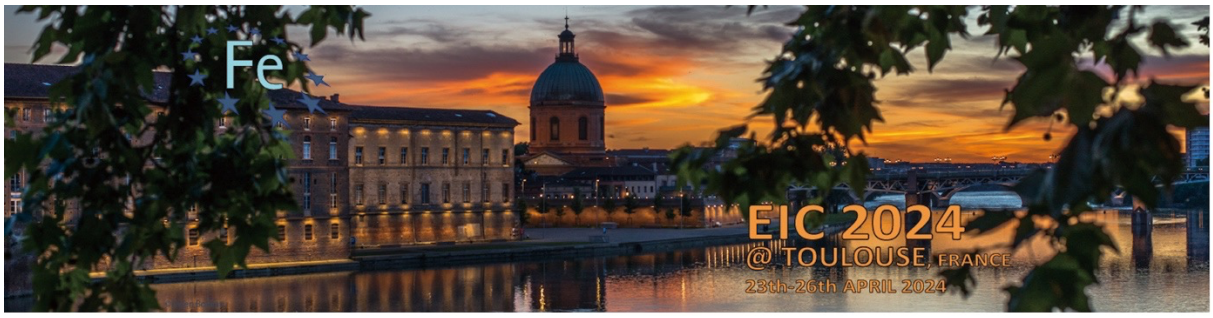
Background: Iron status may influence susceptibility to infection since pathogens require iron to proliferate and iron is critical for host immunity. However, causality is difficult to establish due to confounding bias and reverse causality in epidemiological studies. In this study, we applied Mendelian randomization (MR) to infer whether iron status is causally associated with the risks of severe infections.

Methods: To identify genetic variants for use as instrumental variables (IVs) in MR analyses, we conducted a genome wide association study (GWAS) of iron status in 3928 children in five African countries. We then identified the IVs in large case-control GWAS studies of severe malaria (n=7957 cases and 7746 controls), bacteraemia (1970 cases and 4013 controls), and tuberculosis (3525 cases and 3424 controls) and performed MR analyses.

Results: We found that African-specific genetic loci influenced iron status and risk of severe infection. *GTF3C5*, a locus implicated in transferrin endocytosis, was associated with a 12% and 15% reduced risk of severe malaria and bacteraemia, respectively. *SDR16C5*, a locus involved in retinol metabolism influenced hepcidin levels and a unit increase in genetically determined hepcidin level was associated with 33% protection against overall bacteraemia and 78% protection against Klebsiella pneumoniae bacteraemia. The *FREM3* locus, within the malaria protective Dantu region altered soluble transferrin receptor (sTfR) levels, and a unit increase in genetically determined sTfR level was associated with 70% and 37% protection against severe malaria and bacteraemia, respectively. ***Conclusion***: MR analyses suggest an important link between iron status and severe infection in African children.



THE ROLE OF RIPK1 IN PREVENTING MALARIA-INDUCED NEUROINFLAMMATION

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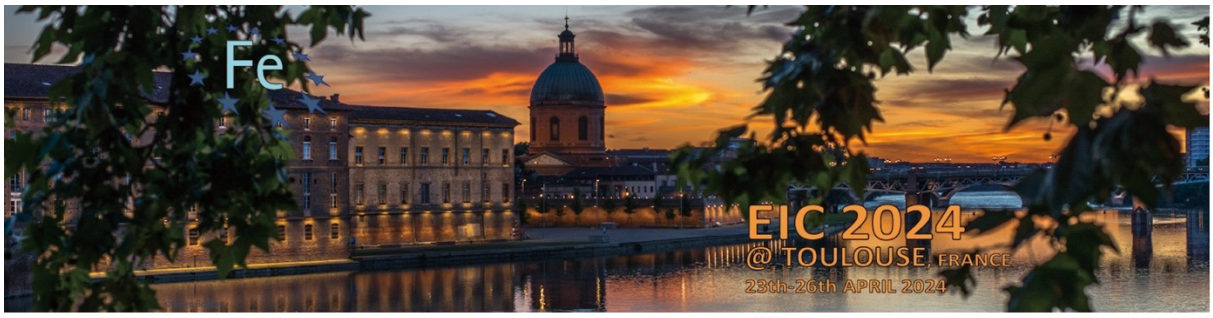
Malaria is a hemolytic disease responsible for high morbidity and mortality rates, particularly in Sub-Saharan Africa. Among the complications, severe malarial anemia (SMA) is a threatening condition. Our data showed that SMA induces brain damage by causing neuroinflammation and iron (Fe) accumulation, presumably due to the release of heme into circulation, which is triggered by parasite replication. In agreement, the exogenous administration of heme in mice was shown to enhance the permeability of the blood brain barrier, fostering neuroinflammation. This effect was also observed upon malaria, regardless of parasites' ability to trigger cerebral or severe forms of malaria. Given that necroptosis underlies the severity of malaria-induced tissue damage, we assessed whether the neuroinflammation, developed during SMA and caused by heme cytotoxicity, is regulated by RIPK1. By using knock-in mice with an inactivate kinase activity, Ripk1S25D, our data demonstrated that those animals were more resistant to the pro-inflammatory priming to the brain caused by the infection, in relation to wild-type controls. The pronounced activation of the immune response in Ripk1S25D infected mice contrasted with the absence of neuroinflammation, as assessed by the significant reduction of brain infiltrated lymphocytes. Similar data were also obtained when injecting these animals with heme, used to mimic the damage induced by hemolytic infections, like malaria. A reduced brain Fe accumulation was found in both infected and heme-injected Ripk1S25D mice, when compared to controls. Overall, our data indicate that the inhibition of necroptosis could become a new therapeutic approach to prevent SMA from causing brain complications.



IRON ABSORPTION, LOSSES AND GAINS IN VIRALLY-SUPPRESSED HIV+ CHILDREN

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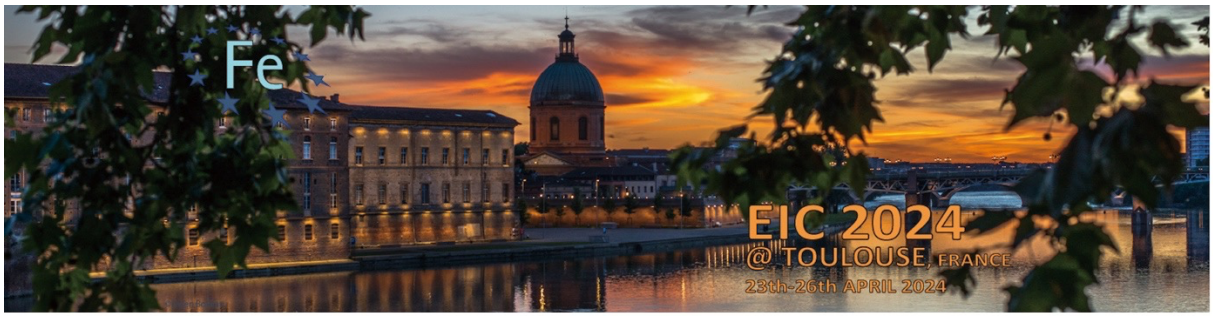
Background HIV+ children are often iron deficient, but the reasons for this are unclear. In HIV+ children, persisting low-grade inflammation may impair iron absorption and/or increase iron losses.

Objective In HIV+ children compared to children without HIV (HIV-), we measured iron absorption from iron fortificants and supplements, and iron absorption and losses from the habitual diet over 6 months. In HIV+ children, we assessed safety and efficacy of oral iron supplements with and without prebiotic galacto-oligosaccharides (GOS).
Design In Study 1, in iron-deficient 8-13 y-old HIV+ children and HIV- children (n=90), we measured iron absorption from maize porridge, a lipid-based nutritional supplement (LNS) and an oral iron supplement. In Study 2, we assessed dietary iron absorption and losses over 6 months in the children (n=90) by stable isotope dilution. In Study 3, we performed a 12-week randomized controlled trial comparing oral iron supplements (50mg/day) without and with 7.5g GOS in HIV+ children (n=83).
Findings At baseline, the HIV+ group was more iron-deficient (P<0.02) and had more inflammation (P<0.01). In the HIV+ group, habitual dietary iron absorption was 34% lower (P<0.001) and iron gains were 20% lower (P<0.05). In Study 3, the group receiving the iron+GOS had greater iron gains (P<0.02) and less enterocyte damage (P<0.05), compared to iron alone.
Interpretation Virally-suppressed HIV+ children have greater inflammation and lower habitual dietary iron absorption and lower iron gains. However, their absorption of oral iron supplements is comparable to HIV- children and providing oral iron with prebiotics may improve efficacy and safety.



LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECS) CONSTITUTE A MAJOR ROUTE FOR HEMOGLOBIN CLEARANCE

ZURAWSKA Gabriela [1], SAS Zuzanna [2], **JONCZY Aneta [1]**, MAHADEVA Raghunandan [1], SLUSARCZYK Patryk [1], CHWALEK Marta [1], KULECKA Maria [3], RUMIENCZYK Izabela [3], JASTRZEBSKI Kamil [1], MIKULA Michal [3], ETZERODT Anders [4], SERWA Remigiusz [5], MIACZYNSKA Marta [1], RYGIEL Tomasz P. [2,6], MLECZKO-SANECKA Katarzyna [1]

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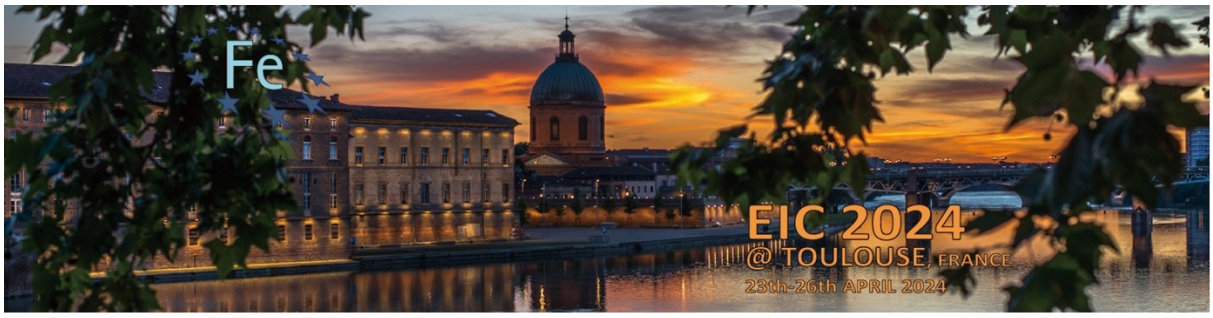
Mild hemolysis of senescent erythrocytes occurs physiologically in the spleen, resulting in hemoglobin (Hb) release, whereas pathologic erythrocyte rupture characterizes several diseases. Iron recycling from Hb and Hb detoxification have been attributed to the sequestration of Hb-haptoglobin complexes by macrophages. However, it remains unclear whether other routes of Hb clearance exist. Here, we identified LSECs as the primary cells responsible for Hb sequestration, a process that involves macropinocytosis and operates independently of the Hb-haptoglobin receptor CD163. We found that LSECs show high iron content and proteomic signatures indicative of adaptation to Hb turnover, hallmarked by high protein levels of HO-1, BLVRB, ferritins, and hepcidin-controlled ferroportin. Erythrocyte transfusion assays further demonstrated that while splenic red pulp macrophages are adept at erytrophagocytosis, liver Kupffer cells and LSECs mainly clear erythrocyte ghosts and Hb, respectively, transported from the spleen via the portal circulation. Moreover, Hb subunits were classified as top-upregulated proteins in LSECs after stressed erythrocyte injection in mice. High-dose Hb injections in mice resulted in transient hepatic iron retention and early activation of the gene encoding HO-1 (Hmox1) in LSECs. This response was associated with the transcriptional induction of the iron-sensing angiokine Bmp6, culminating in hepcidin-mediated transient serum hypoferremia. Injection of Hb and iron citrate elicited distinct transcriptional signatures in LSECs, and the Bmp6 induction was phenocopied by erythrocyte lysis upon phenylhydrazine. Collectively, we propose that LSECs provide a key mechanism for Hb clearance, establishing the spleen-to-liver axis for physiological iron recycling from Hb and contributing to heme detoxification during hemolysis.



IRON CHAPERONE POLY C-BINDING PROTEIN 1 REGULATES IRON SENSING IN LIVERSINUSOIDAL ENDOTHELIAL CELLS

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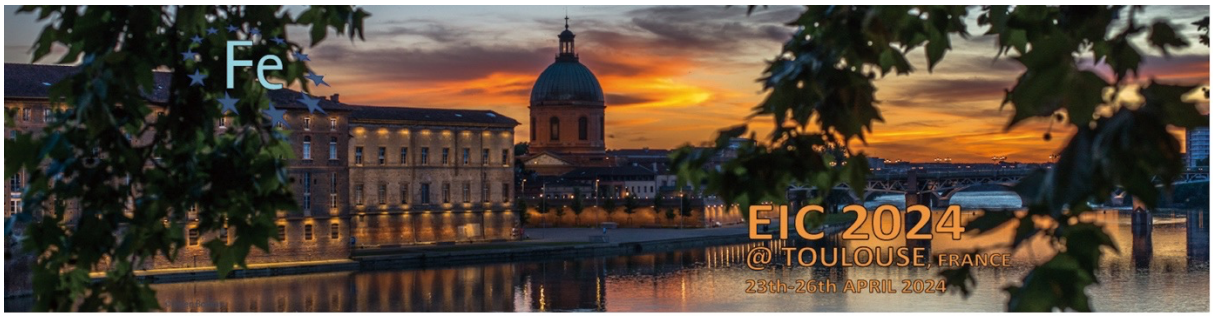
Iron is indispensable for humans, yet is toxic in excess. The mechanisms of precise iron regulation are partially understood and how systemic iron levels are sensed remains unclear. Hepcidin, secreted by hepatocytes, is the master regulator of dietary iron absorption and systemic iron balance in response to iron sufficiency. Hepatocytes are not the site of iron sensing, however. Sinusoidal endothelial cells of the liver (LSECs) sense systemic iron levels and transcriptionally activate expression of the iron-regulatory, bone morphogenetic proteins BMP6 and BMP2. Secretion of these proteins from LSECs triggers hepcidin expression in adjacent hepatocytes. The mechanisms by which LSECs sense iron are not completely known. Oxidative stress caused by iron overload can activate NRF2-mediated BMP transcription, but the activator of BMPs under conditions of physiologic iron balance remain to be identified. We hypothesize that the major iron chaperone of mammalian cells, poly C-binding protein 1 (PCBP1), plays a role in the sensing of iron in the LSECs. PCBP1 is required for binding and intracellular trafficking of chemically reactive iron in mammalian cells and tissues. Here we have constructed a LSEC-specific, mouse model of PCBP1 deletion and preliminary data indicate dysregulation of dietary iron uptake with iron deficiency and microcytic anemia. Iron deficiency was associated with inappropriately high expression of BMPs in the LSEC and high levels of hepcidin production in liver. NRF2 is not activated in the PCBP1-deleted LSECs, demonstrating PCBP1 to be a novel component in the BMP6 and hepcidin regulatory system, thereby contributing to systemic iron homeostasis.



THE FXNI151F MOUSE MODEL OF FRIEDREICH ATAXIA PRESENTS TISSUE-DEPENDENTIRON HOMEOSTASIS ALTERATIONS

PAZOS Maria [1], MEDINA-CARBONERO Marta [1], SANZ-ALCÁZAR Arabela [1], PORTILLO-CARRASQUER Marta [1], DELASPRE Fabien [1], CABISCOL Elisa [1], ROS Joaquim [1], TAMARIT Jordi [1]

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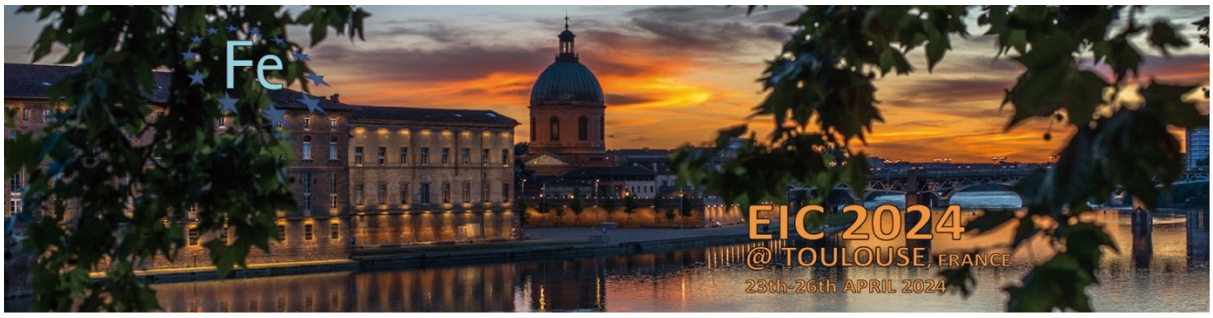
Friedreich Ataxia (FA) is a rare genetic cardio-neurodegenerative disease caused by mutations in the FXN gene resulting in low levels of frataxin, a mitochondrial protein. While most patients are homozygous for a GAA triplet expansion in the first intron of the FXN gene, 5% are compound heterozygous for a GAA expansion in one allele and a point mutation in the other allele. It is well-established that frataxin-deficient mammals present deregulated iron homeostasis, mitochondrial dysfunction and increased oxidative stress. Nevertheless, the tissue-specificity of these alterations has not been analysed in detail due to the lack of suitable animal models. Our lab has recently developed a new FA mouse model based on the human pathological point mutation I154F. It presents low frataxin content in all the tissues and neurobehavioral defects resembling FA patients. Therefore, it is the first model which allows the study of the systemic consequences of frataxin deficiency on iron homeostasis. In this regard, we have observed an increase in total iron content in the nervous system and the liver of FXNI151F mice. Moreover, Iron Regulatory Protein 1 (IRP1) content is decreased in these tissues, while heart presents normal iron and IRP1 levels, and increased IRP2 content. Remarkably, in the liver, IRP1 decrease precedes iron accumulation and iron-sulfur loss. We conclude that frataxin deficiency causes tissue-specific alterations in iron homeostasis. In the liver, reduction of IRP1 content may disturb iron sensing and affect the control of systemic iron metabolism, contributing to anomalous iron distribution among tissues.



Iron-deficiency causes aspartate-sensitive metabolic dysregulation in CD8+ T-cells

TEH Megan [1], GUDGEON Nancy [2], FROST Joe [1], SINCLAIR Linda [3], SMITH Alastair [4], MILLINGTON Christopher [4], KRONSTEINER Barbara [5], ROBERTS Jennie [6], MARZULLO Bryan [6], PRESTON Alexandra [1], REHWINKEL Jan [1], MILNE Thomas [4], TENNANT Daniel [6], DUNACHIE Susanna [5], ARMITAGE Andrew [1], DIMELOE Sarah [2], DRAKESMITH Hal [1]
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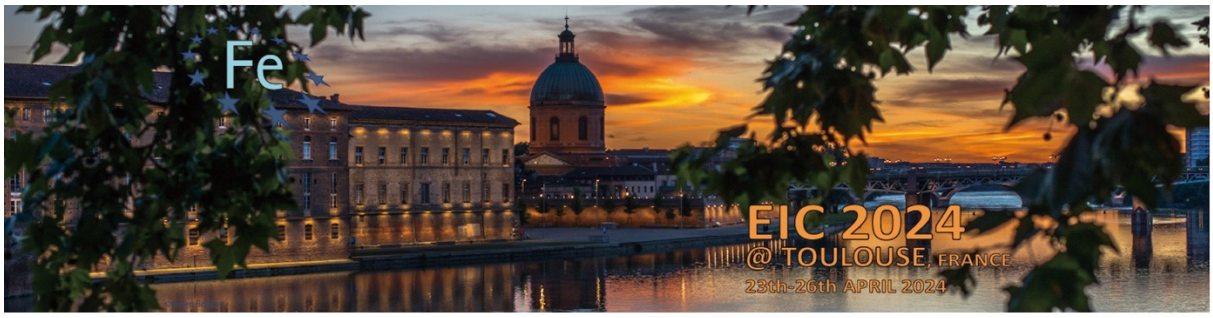
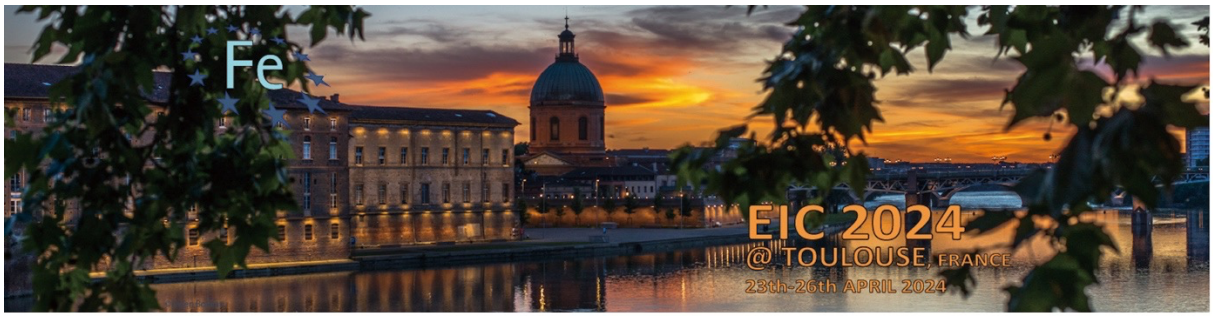
Iron is an essential micronutrient which interacts with ~400 human proteins involved in processes including mitochondrial metabolism, epigenetic regulation and DNA synthesis. Meanwhile, iron deficiency affects >1.2 billion people and is associated with anaemia and suppressed immune responses. However, the biochemical mechanisms underlying these effects remain unclear. Using CD8+ T-cells as a model of normal proliferating cells, we investigated how low iron availability influences cellular biochemistry. Iron starvation reduced cell proliferation, induced the P53 cell cycle arrest pathway and suppressed genes involved in mTORC1 and MYC signalling indicative of aberrant metabolic rewiring. The metabolic dysfunction appeared largely mitochondrial with iron starved cells featuring increased mROS generation and reduced mitochondrial membrane potential suggestive of ETC dysfunction. TCA cycle progression was impaired at the iron dependent enzymes ACO2 and SDH, depleting downstream metabolites including α-ketoglutarate. H3K27me3, a repressive histone mark removed by the iron and α-ketoglutarate dependent KDM6A/B enzymes during T-cell activation, significantly accumulated in iron deprived cells indicating that iron-deficiency impairs epigenetic reprogramming. Despite TCA cycle dysfunction, aspartate, produced downstream of the TCA cycle, was unexpectedly increased in iron restriction while nucleotide precursors downstream of aspartate incorporation were significantly depleted suggesting reduced aspartate usage. Exogenous aspartate substantially rescued the proliferation of iron deprived cells suggesting that endogenous aspartate sources are unusable, possibly due to mitochondrial aspartate trapping. Overall, iron deficiency results in a profound mitochondrial dysfunction resulting in impaired aspartate utilisation. This work provides insight as to how metabolic and iron modulatory interventions could be coupled to augment or suppress immunity.



COA DEFICIENCY ASTROCYTE MODEL HIGHLIGHT THE ROLE OF MITOCHONDRIAL IRON TRIGGERING NEURODEGENERATION

SANTAMBROGIO Paolo [2], COZZI Anna [2], RIPAMONTI Maddalena [1,2], BERNO Valeria [2], CAMMEROTA Eugenia [2], MORO Andrea [1,2], BALESTRUCCI Chiara [1,2], PELAGATTI Alessio [2], RUBIO Alicia [3], **LEVI Sonia [1,2]**
[1] Vita-Salute San Raffaele University, Milano, Italy; [2] IRCCS-San Raffaele Scientific Institute, Milan, Italy; [3] Institute of Neuroscience, National Research Council, Milan, Italy

We utilized hiPS-derived astrocytes of PANK2- and COASY-associated neurodegeneration (PKAN and CoPAN), two Neurodegeneration with Brain Iron Accumulation (NBIA) disorders caused by mutations in genes that codify for key enzymes in Coenzyme A biosynthetic chain reactions, to investigate the relationship between iron mitochondrial impairment and neuronal death. Both diseases are characterized by progressive neurodegeneration and a huge iron-accumulation in the globus pallidus of patients. A neurotoxic feature of PKAN astrocytes was already demonstrated in co-cultures experiments. Both PKAN and CoPAN astrocytes showed cytosolic iron accumulation, alteration of iron metabolism and mitochondria morphology, and were prone to develop a stellate-like phenotype. The tendency to stellation seems to be correlated with the amount of up-taken transferrin, suggesting a potential impairment in membrane dynamics that could be at the basis of iron overload. Indeed, experiments aimed to analysis of constitutive exo-endocytosis led to the finding of a general impairment in the constitutive endosomal trafficking in PKAN astrocytes. Super-resolution microscopy experiments showed that a significantly lower number of transferrin-enriched vesicles were in contact with mitochondria in PKAN, thus confirming an impaired intracellular fate of cargo endosomes. Analysis of mitochondrial iron homeostasis and tubulin feature reveal that: i) mitochondria display an iron deficiency status caused by an impairment of iron deliver to mitochondria due to alteration of tubulin acetylation ii) mitochondrial iron deficiency cause cytosolic iron overload due to the restriction of ISC biosynthesis that affect iron-protein regulation. This highlighted the mitochondrial main role in iron homeostasis and its involvement in pathogenesis of CoA deficiency.



maternal iron deficiency impairs postpartum reversal of pregnancy-related cardiac remodelling.

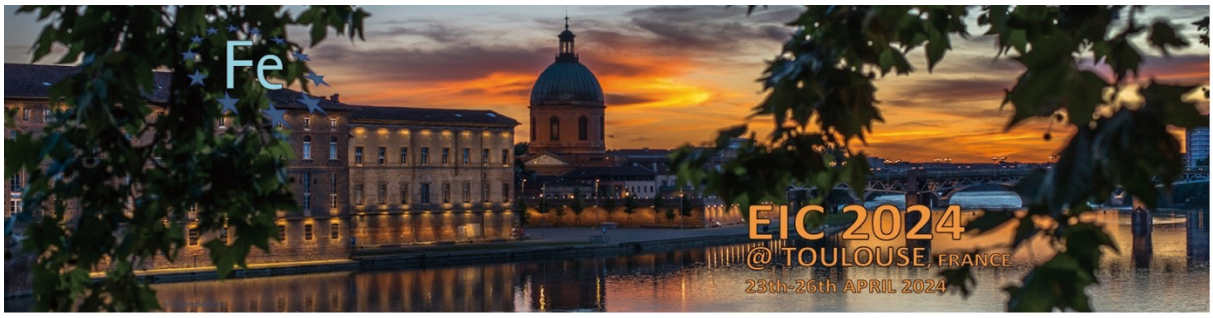
VERA-AVILES Mayra [1], KABIR Syeeda [1], LAKHAL-LITTLETON Samira [1]
[1] University of Oxford, Oxford, United Kingdom

Background-
The maternal heart remodels during pregnancy to adapt to increased haemodynamic load. This involves hypertrophy of the left ventricle. The maternal heart reverse-remodels back to pre-pregnancy state rapidly in postpartum. Inadequate reverse-remodelling of the maternal heart is now emerging as a precursor to heart failure. However, the risk factors that impair reverse remodelling are not fully understood, The aim of his study is to explore the role of maternal iron status in maternal heart adaptations during pregnancy and postpartum.

Methods
We placed wild type female mice on iron-replete (200ppm) or iron-deficient diets (5ppm) for 10 weeks prior to mating. They were maintained on these diets throughout pregnancy and postpartum. We examined heart function by Cine MRI, and quantified myocardial and systemic iron levels.

Results
In both iron-replete and iron-deficient mothers, the hearts responded to pregnancy by increasing LV mass, LV end diastolic and end-systolic volumes. By 3 weeks postpartum, the hearts of iron-replete mothers had returned to pre-pregnancy levels. However, the hearts of iron-deficient mothers remained hypertrophied. This was preceded by profound depletion of systemic and myocardial iron, myocardial upregulation of the iron exporter ferroportin, and suppression of hepcidin.

Conclusion
These data identify maternal iron deficiency and in particular myocardial iron depletion as a risk factor for impaired postpartum reverse remodelling, and as such a new female-specific risk factor for heart failure. These findings have the potential to improve maternal outcomes, because therapies that replenish systemic and myocardial iron rapidly are available.



HEPCIDIN CHANGES IRON STATUS AND PREDICTS LONG-TERM OUTCOMES FOLLOWING MYOCARDIAL INFARCTION

KABIR Syeeda Nashitha [1], VERA-AVILES Mayra [1], MOHAMMAD Goran [1], UELAND Thor [2], LAKHAL-LITTLETON Samira [1]
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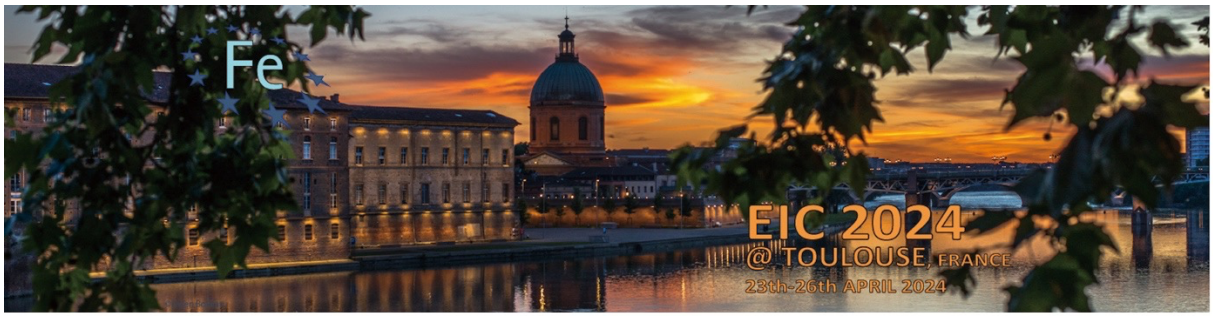
Introduction: Myocardial infarction (MI) is followed by acute changes in serum iron markers. The mechanisms behind this and its long-term impact are unknown. This work aims to explore the role of hepcidin in this context.

Methodology: We conducted a clinical study nested within a placebo-controlled randomised trial of IL6 receptor antagonist Tocilizumab in patients with ST-elevation-MI (CT03004703). Changes in heart function, hepcidin, serum iron markers and inflammatory markers were measured. In mice, MI was induced surgically. Heart function was analysed by MRI, serum iron-markers, and myocardial labile iron quantified using iron-caged luciferin.

Results: In patients receiving placebo (n=98), hepcidin rises acutely within 24 hours post-MI ($p = 0.0003$) while serum iron markers drop ($p = 0.014$). This did not occur in the Tocilizumab group (n=100). Hepcidin levels at 24 hours post-MI correlated with the size of the area at risk ($p = 0.032$) and predicted elevated NTproBNP ($p = 0.007$), larger infarct size ($p = 0.001$), and lower LVEF % ($p = 0.029$) at 6 months, even after correcting for confounders. In mice, hepcidin levels also rose acutely post-MI, and this was accompanied by increased myocardial LIP in the area at risk.

Conclusions: Hepcidin rises acutely post-MI, deriving from the myocardial area at risk, under the influence of IL-6. These changes are accompanied by a drop in serum iron and predict adverse cardiac remodelling. Evidence from the mouse model suggests the impact of hepcidin on adverse cardiac remodelling may relate to excess labile iron retention within the area at risk.



EXCHANGE PROTEIN DIRECTLY ACTIVATED BY CAMP 1, PROMOTES IRON-MEDIATED CARDIOMYOCYTE DEATH

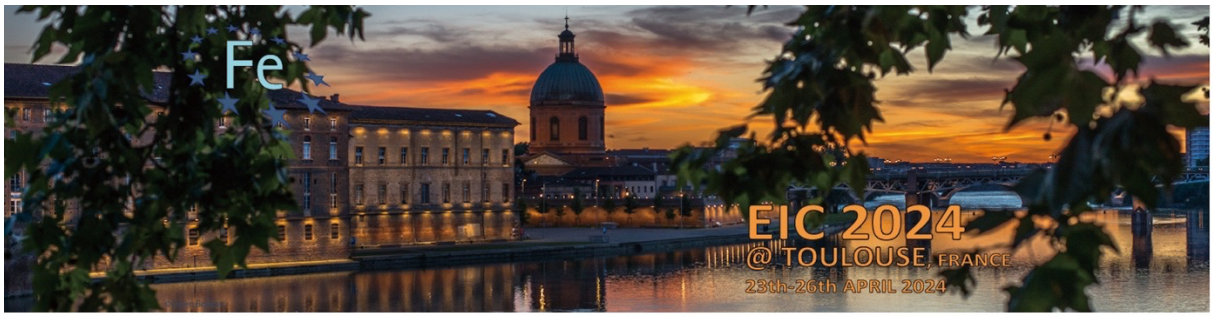
AGBEGBO Ezechiel [1], HAIDAR Fraha [1], FORMOSO Karina [1], SANTIN Yohan [1], DETRAIT Maximin [1], LEZOUALCH Frank [1]
[1] INSERM UMR 1297, TOULOUSE, France

Iron-overloading conditions promotes cardiac dysfunction and failure. Ferroptosis is a new form of regulated cell death that is characterized by iron overload, leading to the accumulation of lethal levels of ROS and lipid peroxidation. Our previous RNA-Sequencing analysis performed in neonatal rat ventricular myocytes (NRVMs) showed that pharmacological modulation of ***Exchange protein directly activated by cAMP 1 (EPAC1) regulated gene markers of ferroptosis*** such as transferrin receptor (tfrc), soluble carrier 40A1 (slc40a1), Acyl-CoA synthetase long chain family member 4 (acsl4).

This study aims to determine the role of EPAC1 in cardiomyocyte iron metabolism and its implication in iron-mediated cell death of cardiomyocytes.

As expected, a known ferroptosis inducer, erastin induced concentration-dependent cardiomyocyte death, as assessed by LDH release. Consistently, micromolar concentrations of erastin promoted ROS and lipid peroxidation accumulation in NRVMs. To further confirm a potential impact of EPAC1 protein in erastin-induced ferroptosis cell death, we used a pharmacological EPAC1 inhibitor, AM-001. Analysis of cell viability revealed that AM-001 markedly prevented erastin-induced cell death in NRVMs. Accordingly, we found that the increase in ROS levels and accumulation of lipid peroxides after erastin treatment were prevented in NRVMS pretreated with AM-001 (20 µM). Similarly, iron accumulation, ROS as well as cell death were prevented in NRVMs treated with ferric ammonium citrate (100 µM) and AM-001 (20 µM).

Altogether our data show that EPAC1 blockade prevents iron-induced cell death in NRVMs. These results will further be confirmed in *in vivo* mouse models of cardiomyopathy induced by doxorubicin or iron dextran.



Oral Presentations session | OP-07 - Hepcidin regulation

AUTOCRINE FUNCTION FOR HEPCIDIN IN KUPFFER CELLS DURING IRON OVERLOADING

BLANCHET Margault [1], BENBIDA Cheïma [1], DESSENA Mattia [1,3], PEYSSONNAUX Carole [2], GAUTIER Emmanuel [1], HUBY Thierry [1]

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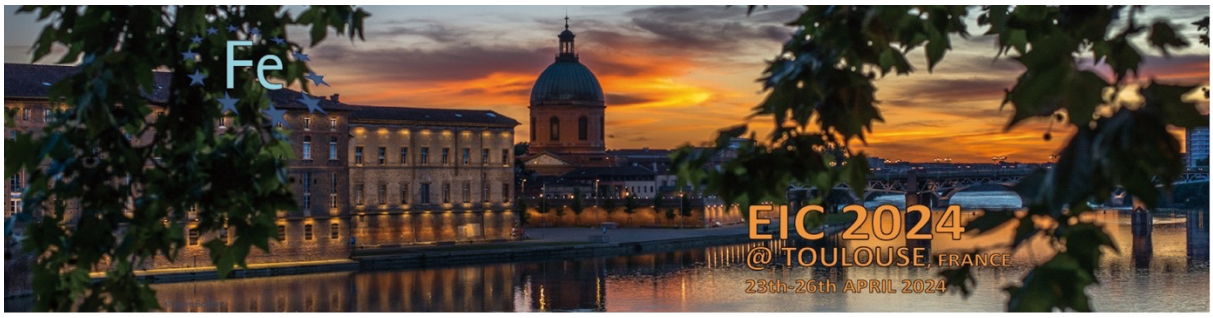
Iron metabolism is tightly regulated so iron is available for biological functions while preventing its cytotoxic effects. Macrophages play a central role in establishing this balance. Kupffer Cells (KCs), liver resident macrophages, are involved in the elimination of damaged erythrocytes, ensuring systemic iron recycling to prevent damage from excess iron deposition in organs. Moreover, the hepcidin (*HAMP*)/ferroportin axis is critical to coordinate cellular iron export. Hepcidin induces the degradation of the iron exporter ferroportin, suppressing iron release from macrophages and parenchymal cells. The hepatocyte-derived hepcidin control on systemic iron homeostasis is well-known, but the autocrine role of hepcidin produced by the macrophage remains to be more defined. Here, we observed that iron overloading by iron dextran injection into wild-type mice resulted in a rapid and partial loss of embryonically-derived KCs (EmKCs composing the KC pool in healthy livers). Concomitantly, monocyte-derived macrophages were recruited to the liver and acquired the prototypical KC marker CLEC2. However, engraftment of these monocyte-derived KCs was only transient, and EmKCs proliferated to replenish the KC pool later. Similar studies of iron overloading were replicated in mice with hepcidin deficiency in macrophages ($Hamp^{MacKO}$; Lysm-Cre x $Hamp^{lox/lox}$), including KCs. Decreased EmKCs density was rapidly observed in both $Hamp^{MacKO}$ and $Hamp^{lox/lox}$ control mice following iron dextran injection. However, $Hamp^{MacKO}$ mice failed to restore their EmKCs pool overtime, and associated with a reduced proliferation rate. These preliminary observations suggest that hepcidin plays a role in Kupffer Cells response to iron overloading.



Role of Par2 and macrophages in hepcidin regulation

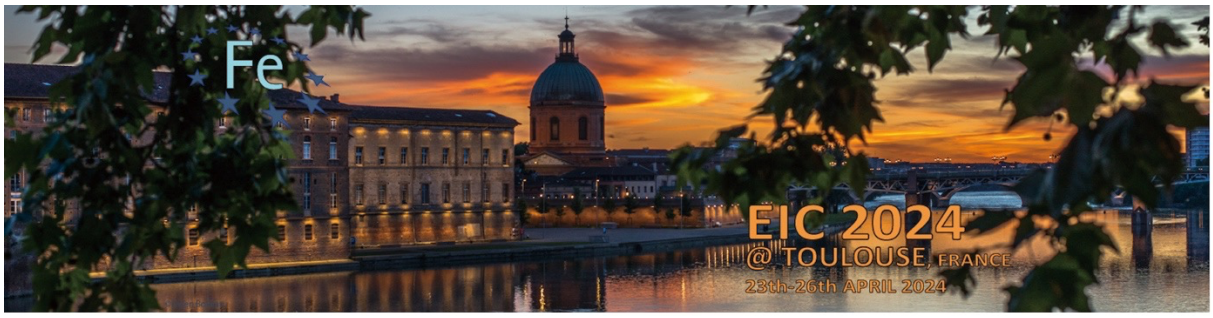
BERGER Mathieu [1], BELOT Audrey [1], LATOUR Chloé [1], PALIN Anaïs [1], BESSON-FOURNIER Céline [1], BASSO Lilian [2], VERGNOLLE Nathalie [1], HÉLÈNE Coppin [1], ROTH Marie-Paule [1], CAMERER Eric [3], DERAISON Céline [1], MEYNARD Delphine [1]

[1] INSERM U1220 Institut de recherche en santé digestive, Toulouse, France; [2] INSERM U1291 Institut Toulousain des Maladies Infectieuses et Inflammatoires, Toulouse, France; [3] INSERM U970 Paris Cardiovascular Research Center, Paris, France

During erythropoietic stress, inhibition of hepcidin increases the bioavailability of iron in parallel to hemoglobin synthesis. The secretion of erythroferrone by erythroblastic progenitors and the production of the hepatokine Fgl1 have previously been implicated in hepcidin suppression. However, we have observed that, in addition to these factors, suppression of hepcidin in response to erythroid stress is also dependent on PAR2, a protease-activated receptor.

In this study, we highlight the role of PAR2 in regulating hepcidin under conditions of erythroid stress. First, we observed that Par2-/- embryos are anemic. Then, to investigate its role in hepcidin suppression, we phlebotomized Par2-/- mice and analyzed hepcidin expression 48 hours later. We observed that Par2 deletion prevents the suppression of hepcidin. Since Par2 is not expressed in hepatocytes, we suspected a role in another cell type. Given that Par2 plays an important role in macrophages, we generated mice with a specific deletion of Par2 in macrophages by crossing Par2-floxed mice with LysM-cre mice and subjected them to phlebotomy. Similarly, to Par2-/- mice, Par2 deletion in macrophages prevents hepcidin suppression. We then questioned the essential role of macrophages in erythroid stress-dependent hepcidin suppression using mice expressing the diphtheria toxin receptor only in macrophages (LysM cre iDTR). In this mouse model, when macrophages are depleted hepcidin is not suppressed in response to phlebotomy. These data highlight a new role of macrophages in the regulation of hepcidin.

We are now characterizing the roles of macrophages and Par2 in this cell type in the regulation of hepcidin.



Osteoblast-selective Erfe Deletion Confirms Cell-Autonomous Role of ERFE in the Bone homeostasis

VAN CALOEN Gabrielle [1], NA PHATTHALUNG Pinanong [1], PLANOUTENE Marina [1], GUMEROVA Anisa [1], WITZTUM Ronit [1], INGBER Eva [1], SULTANA Farhath [1], KORKMAZ Funda [1], LEVY Maayan [1], YUEN Tony [1], ZAIDI Mone [1], **GINZBURG Yelena [1]**

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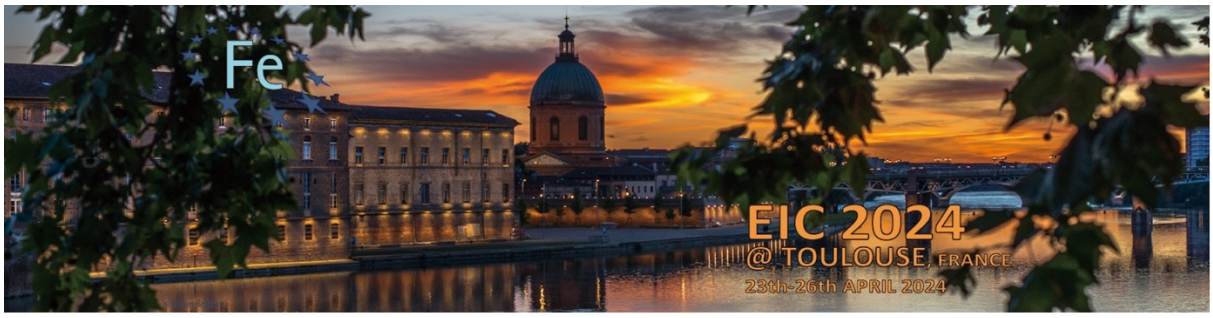
We explore the incompletely understood crosstalk between erythropoiesis, bone homeostasis, and iron metabolism. We anticipate that, because erythroferrone (ERFE) acts by sequestering bone morphogenetic proteins (BMPs) to inhibit the main signaling pathway in hepcidin regulation and BMPs are crucial for bone modeling and remodeling, ERFE plays a central role in this mutual regulation. We discovered that osteoblasts express Erfe mRNA and functional ERFE is found in cultured osteoblast supernatants. Erfe-/- mouse osteoblasts displayed enhanced mineralization and osteoblastogenesis. However, skeletal phenotyping revealed that Erfe-/- mice had low bone mineral density. We thus generated conditional Erfe knockout (Erfe floxed) mice and analyzed progeny crossed with osteoblast-selective Cre (Col2.3-Cre). Our results demonstrate that Erfe expression is selectively lost in osteoblasts of Col2.3-Cre;Erfefl/fl mice but not erythroblasts and Col1a1 and Alp expression is increased in cultured osteoblasts from Col2.3-Cre;Erfefl/fl mice, consistent with enhanced osteoblast differentiation. First, Col2.3-Cre;Erfefl/fl mice do not exhibit decreased bone mineral density. Next, parathyroid hormone treated Col2.3-Cre;Erfefl/fl mice demonstrate enhanced bone mineral density relative to Erfefl/fl mice, consistent with an advantage during bone formation when ERFE is lost selectively in osteoblasts. As expected, no differences in spleen weight, hemoglobin, reticulocyte counts, and hepcidin expression are evident between Erfefl/fl and Col2.3-Cre;Erfefl/fl mice. Lastly, Col2.3-Cre;Erfefl/fl mice do not suppress hepcidin in response to phlebotomy. Taken together, our results demonstrate the utility of the newly generated Erfefl/fl mice, supports our hypothesis that cell-autonomous osteoblast ERFE expression is relevant in bone homeostasis, and confirms a possible systemic contribution to canonical ERFE function.



LOCALLY PRODUCED ARTICULAR HEPCIDIN IS ASSOCIATED WITH RHEUMATOID AND PSORIATIC ARTHRITIS

LAMALLE Camille [1], KARIM Zoubida [1], DEGBOÉ Yannick [1,2,3]

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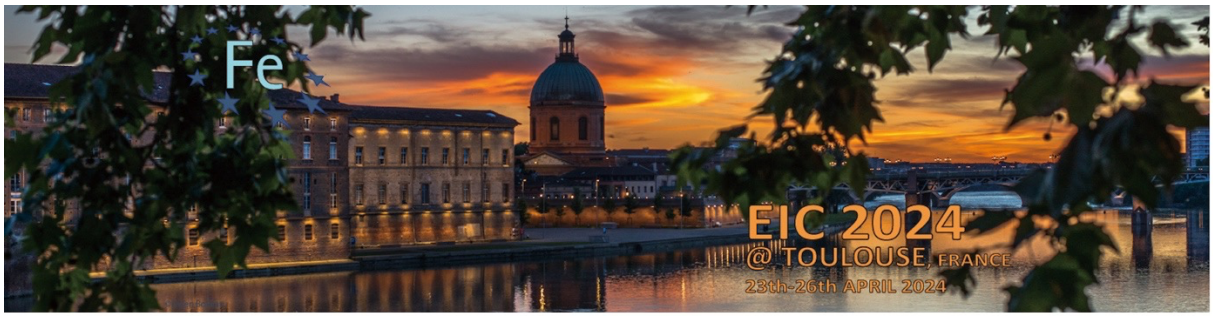
Arthritis is a joint inflammation associated with chronic autoimmune rheumatism such as rheumatoid arthritis (RA) and psoriatic arthritis (PsA). The most common form, RA, is characterized by synovial inflammation, cartilage and bone destruction. Recently, several studies have identified the involvement of local extra-hepatic hepcidin production in several inflammatory phenomena. However, how local hepcidin influences articular physiology and contributes to arthritis is poorly understood.

Our aim was to explore the local articular production of hepcidin and to evaluate its role in RA and PsA.

Using a cohort of arthritis patients, we measured (LC/MS/MS) higher levels of hepcidin in arthritis synovium, compared to osteoarthritis controls. To confirm a local articular production, we isolated mononuclear cells from arthritis synovial fluids and showed (RT-qPCR) that leucocytes expressed hepcidin mRNA. To recapitulate the conditions of inflammatory synovial infiltrate, we stimulated ex-vivo peripheral blood mononuclear cells with synovial fluids and showed that only inflammatory synovial fluids triggered hepcidin production. IL-6, a well-known pro-inflammatory cytokine in arthritis, is the major regulator of hepcidin in inflammatory conditions. Hepcidin induction by synovial fluids was only partially inhibited with IL-6R inhibitor but totally repressed with Filgotinib, a JAK inhibitor, suggesting additional factors to stimulate articular hepcidin in arthritis. Futures omics analyses will determine the regulatory pathways of hepcidin and iron metabolism in arthritis. Our study strongly suggests that hepcidin production takes place locally in the joint under inflammatory arthritis, providing a state of the art for exploring the involvement of hepcidin and iron in the pathophysiology of RA and PsA.



Oral Presentations session | OP-08 - Iron and liver diseases

Activation of the BMP-SMAD pathway counteracts MASLD and its complications.

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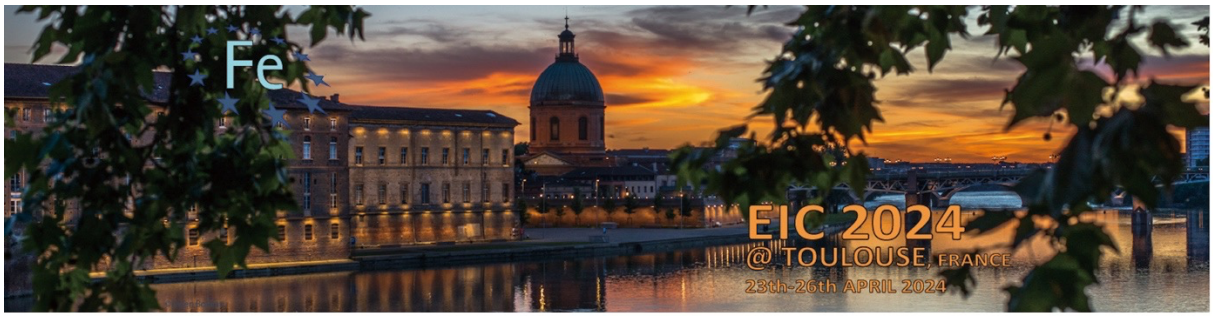
Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of chronic liver disease, which is characterized by fat accumulation and insulin resistance and can progress to steatohepatitis. Excess iron in hepatocytes favors MASLD, suggesting that lipid and iron metabolism are linked. The hepatic BMP-SMAD-pathway controls systemic iron homeostasis. In this context, two inhibitors of the BMP-SMAD-pathway, the TMPRSS6 and the immunophilin FKBP12, have been described. Our unpublished in vitro and in vivo data have shown that upregulation of BMP-SMAD-pathway activates the peroxisome-proliferator-activated-receptor (PPAR)α, suggesting a link between iron and lipid/glucose metabolism. In addition, the expression of key components of BMP-SMAD-pathway as well as hepcidin is reduced, while FKBP12 is upregulated in the liver of MASLD mouse model, suggesting that suppression of hepatic BMP-pathway and altered iron homeostasis are responsible for disease progression.
For these reasons, we hypothesize that activation of the BMP-SMAD-pathway may be a potential therapeutic approach for this disease. To this end, wild-type mice were fed a fructose-palmitate-cholesterol (FPC) diet that induces hepatic steatosis, inflammation and fibrosis, recapitulating the main features of the human disease. When hepatosteatosis was established, the liver BMP-SMAD-pathway was upregulated by antisense oligonucleotides (ASOs) targeting TMPRSS6. Treatment with Tmprss6-ASO counteracts FPC-induced hepatomegaly, ameliorates hepatosteatosis, and reduces hepatic triglyceride accumulation and expression of genes involved in lipid storage and de novo lipogenesis. Moreover, hyperactivity of the BMP-SMAD-pathway decreases the expression of pro-inflammatory cytokines and prevents collagen deposition and fibrosis. Overall, these data show that pharmacological activation of the BMP-SMAD signaling pathway improves MASLD.



INTERPLAY OF HEME AND GLUCO-LIPID METABOLISM: IMPLICATIONS FOR LIVER PATHOPHYSIOLOGY

AMMIRATA Giorgia [1], FIORITO Veronica [1], RONCO Carola [1], RIGANTI Chiara [2], FAGOONEE Sharmila [1], ALTRUDA Fiorella [1], TOLOSANO Emanuela [1]

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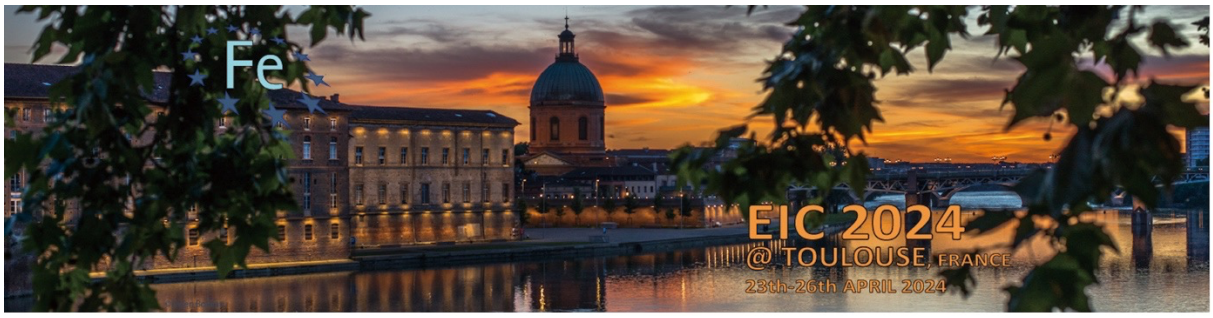
The liver is a pivotal regulator of systemic glucose and lipid levels, serving as a central hub for metabolism. Dysfunctions in hepatic nutrient sensing and processing contribute to the development of metabolic syndrome and progression to non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), two liver pathologies exhibiting sexual dimorphism. The metabolism of iron and of its bioavailable form, heme, are intricately linked to nutrient utilization, responding to nutritional cues such as glucagon during fasting and insulin post-prandially, and influencing the expression of glycolytic and oxidative enzymes.

Our previous works have shown that FLVCR1a, a plasma membrane transporter first recognized as a heme exporter but recently identified as a choline importer, regulates ALAS1-mediated heme synthesis. Our recent findings indicate that deletion of Flvcr1a in hepatocytes (LivKO mice) results in decreased heme biosynthesis and in heme-dependent reduction of glucose uptake and glycolysis, along with elevated fatty acid uptake and oxidation, oxidative phosphorylation and cholesterol production. Based on these results, we hypothesized that the metabolic dysregulation elicited by impaired hepatic heme synthesis in these mice predisposes them to metabolic syndrome. To test this in a gender-specific manner, male and female LivKO mice were subjected to high-fat high-fructose (HFF) diet. Preliminary data indicate exacerbated insulin resistance and compromised gluco-lipid metabolism in LivKO males and females under HFF diet compared to matched controls. These results suggest that rewiring of hepatic heme metabolism contributes to systemic gluco-lipid dysregulation, hinting at heme biosynthesis as a potential target for the prevention/treatment of metabolic syndrome and NAFLD.



ROLE OF FGL1 IN LIVER-RELATED METABOLIC DISEASES

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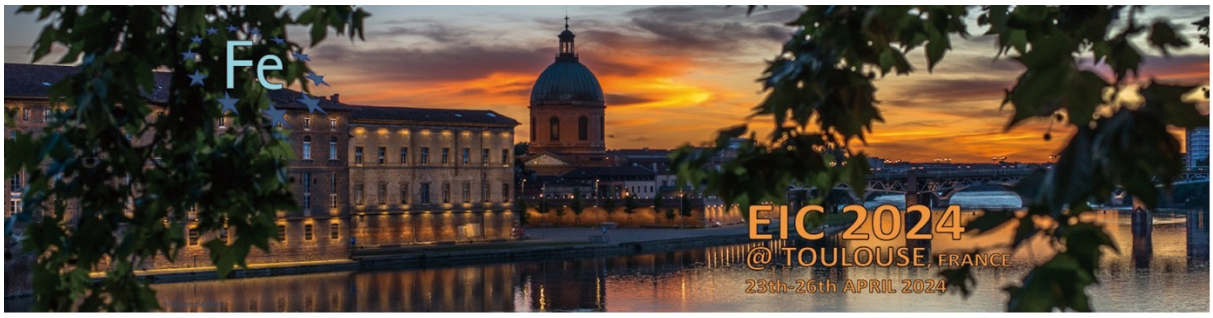
Metabolic associated steatotic liver disease (MASLD) is the hepatic manifestation of the metabolic syndrome that affects 30% of the adult worldwide population. MASLD is a pathological cascade that starts from a benign steatosis and evolves toward nonalcoholic steatohepatitis (MASH) and hepatocellular carcinoma (HCC). While the steatosis and MASH stages are reversible, the cirrhosis stage is irreversible and life threatening. Current treatments against HCC are largely inefficient. A better understanding of the mechanisms involved in this pathological cascade is a prerequisite to envision the development of new therapeutic strategies. **Evidence in the literature indicate that the recently described hepcidin suppressor FGL1 contributes to the progression of steatosis and HCC but its function in the pathogenesis of MASLD is unknown**. We therefore explored the potential contribution of FGL1 to the pathogenesis of liver diseases.

Mice with specific deletion of Fgl1 deletion in hepatocytes (Fgl1-LKO) were fed a western diet for 16 weeks to induce a MASH-like phenotype. Circulating levels of triglycerides, cholesterol, free fatty acids and transaminases were measured at 16 weeks and liver steatosis and fibrosis was analyzed on histological tissue sections. Mice deficient for Fgl1 exhibited impaired glucose tolerance and increased circulating lipids and transaminases concentration.

While the histological analysis is ongoing, these results suggest that FGL1 exerts a protective function against liver injury and may be a relevant therapeutic target for the management of the transition from MASH to HCC.



THE ROLE OF THE HEME-EXPORTER FLVCR1 IN HEPATOCELLULAR CARCINOMA

DUARTE Tiago L [1,2], VIVEIROS Nicole [1], GODINHO Catarina [1,3], LAMAS Sofia [1], LOPES José Manuel [1,4], ABKOWITZ Janis L [5], OLIVEIRA Maria José [1,6], DUARTE Delfim [1,7,8]

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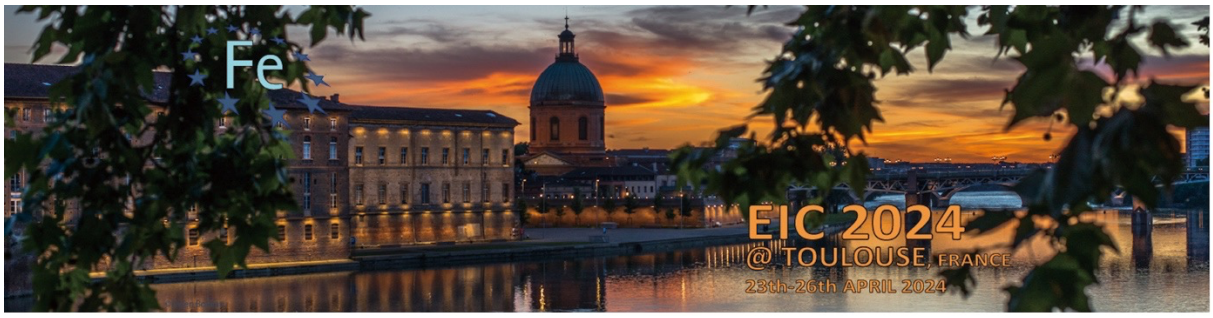
Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver cancer, the third leading cause of cancer-related mortality. There is an unmet need to discover new biological mechanisms in HCC and develop new therapies. The heme exporter FLVCR1 is overexpressed in HCC and associated with poor prognosis, but its role in HCC pathophysiology remains unexplored. We aimed to investigate the role of FLVCR1 in HCC.
To assess the impact of liver-specific *Flvcr1* KO in the development of HCC in vivo, we treated Flvcr1$^{fl/fl}$;alb-cre and Flvcr1$^{fl/fl}$ male mice (N=50 each genotype) with diethylnitrosamine (DEN) and carbon tetrachloride (CCl_4). At the age of 8 months, both groups developed hepatocellular adenomas. Hepatic *Flvcr1* KO did not affect the overall tumor burden (relative liver weight, total tumor volume, largest tumor area) or serum markers of liver injury (AST or ALT activity, bilirubin). However, the incidence of HCC, determined by blind histologic analysis, was significantly reduced in Flvcr1$^{fl/fl}$;alb-cre mice compared to Flvcr1$^{fl/fl}$ animals (13.73% vs 32.69%; p=0.0349).
In parallel, we generated FLVCR1 KO in human HCC cells (Huh7) by CRISPR/Cas9. Consistent with a defect in heme export, FLVCR1 KO Huh7 clones displayed increased levels of intracellular heme. The loss of FLVCR1 function decreased cell migration in vitro (time-lapse wound healing and individual live cell imaging), as well as the ability of Huh7 cells to invade the chorioallantoic membrane (CAM) model, while it potentiated new vessel formation in vivo. Overall, our results suggest that FLVCR1 has a pro-tumorigenic role in HCC.



CONSENSUS CRITERIA FOR METABOLIC HYPERFERRITINEMIA DIAGNOSIS AND GRADE IN A LARGE COHORT WITH MRI

STEPHANT Sophie [1], MASROUR Oumnia [1], LAINE Fabrice [1], MORCET Jeff [1], GANDON Yves [1], **BARDOU-JACQUET Edouard [1]**

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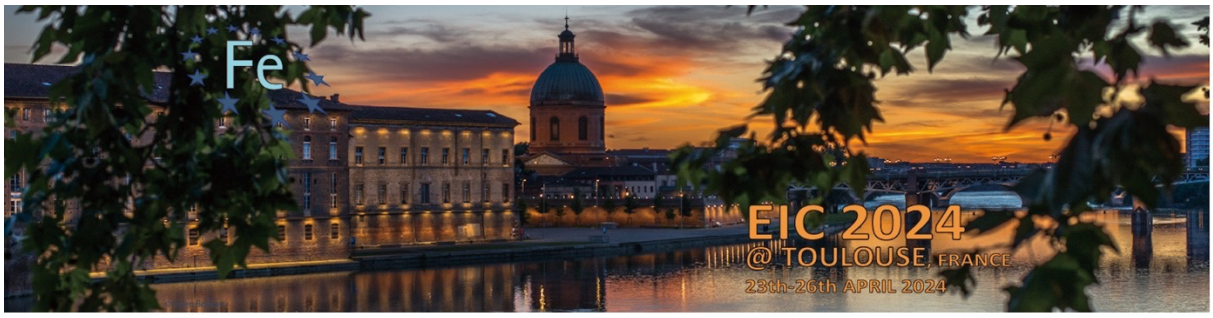
Background:
Recent consensus for Metabolic hyperferritinemia (MHF) has defined diagnosis criteria and grading with cutoff value for ferritin and hepatic iron concentration (HIC). Our aim was to assess their relevance in a large cohort of patients.

Method:
Patients with increased ferritin with available HIC as determined by liver MRI, and fulfilling criteria for MHF according to the recent consensus were included. Multivariate regression analysis was used to identify determinant of HIC and ferritin. ROC curves were used to assess the cut-off suggested.

Results:
534 patients (age:58.6±10.6; 79.8% male, BMI 29.5±4.6) were included. Mean ferritin was 866±655µg/L, and HIC was 93±65µmol/g. Strong correlation between ferritin and HIC was observed (rho=0.70). Multivariate analysis revealed significant associations of ferritin with HIC, ASAT, transferrin saturation (Tsat), triglycerides, and smoking. HIC was associated with ferritin, Tsat, BMI, smoking, ASAT, hemoglobin, age, and alcohol consumption. Grading according to ferritin was 25.3% grade 1, 42.5% grade 2, and 30.9% grade 3. According to HIC grading were 22.3%, 28.3% and 49.4% respectively.

The AUC for ferritin diagnosing HIC≥74µmol/g was 0.83, with a 1000µg/L showing 51% sensitivity, 90% specificity. Patients with Grade 3 MHF according to HIC, were older with lower BMI, higher Tsat, lower ASAT, lower ALT, and lower GGT. Fib4 test results were comparable between MHF grade determined by HIC or ferritin.

Conclusion:
The proposed ferritin and HIC cutoffs effectively stratify MHF severity. However, in our population ferritin tends to underestimate HIC thus emphasizing the importance of integrating both serum ferritin and HIC for comprehensive MHF assessment.



TARGETING THE HEPATIC EPIGENETIC REGULATORS SUV420H1/2 TO COUNTERACT MASLD AND ITS COMPLICATIONS

PAGANI Alessia [1,2], BAVUSO VOLPE Letizia [1], PETTINATO Mariateresa [1,2], ALTAMURA Sandro [3], MALVESTITI Francesco [4], GUO Shuling [5], FURIOSI Valeria [1], CARLEO Rossana [1], NAI Antonella [1,2], VALENTI Luca [4,6], SILVESTRI Laura [1,2]
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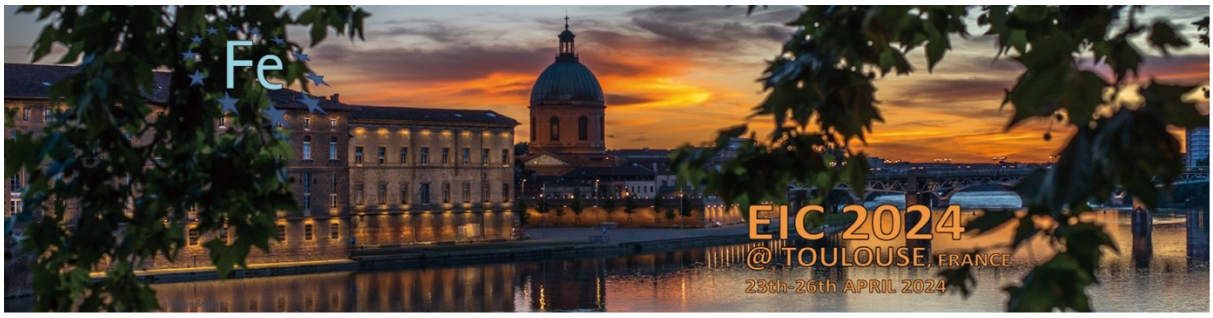
Several factors contribute to MASLD, including impaired iron metabolism, but the molecular mechanisms involved are still unknown. Notably, a genomic region encompassing the histone methyltransferase Suv420h has been associated with iron-dependent hepatic steatosis in mice, suggesting that epigenetic remodeling plays a role in linking lipid and iron metabolism.

To determine whether Suv420h contributes to MASLD, we compared the differentially expressed signaling pathways in the liver of bariatric patients stratified for Suv420h1-h2 expression. We then generated mice lacking Suv420h1 in hepatocytes and h2 in the whole body (dKO) and characterized them after 16 weeks of a NASH-inducing-diet (FPC). In bariatric patients, Suv420h1-h2 positively correlated with inflammatory and IFNγ response, and EMT, independently of other confounders. Additionally, in patients expressing higher levels of Suv420h1-h2, overrepresentation analysis indicated enrichment in genes involved in extracellular matrix, collagen fibril organization and leukocyte migration. In control mice, the FPC diet resulted in mild overweight and hepatomegaly, while dKO were protected and showed less hepatomegaly, fewer lipid droplets, reduced monocyte recruitment, collagen deposition and white adipose tissue hypertrophy. Consistently, liver triglycerides and transaminases were reduced. Liver RNAseq confirmed the protective effect of h1-h2 deletion. FPC-control mice showed increased serum iron and decreased hepcidin. Interestingly, iron parameters normalized in dKO. Pharmacologic targeting of liver h1/h2 by antisense-oligonucleotides in hepatosteatosis confirms their protective role in preventing disease progression.
Suv420h can be considered a promising therapeutic target for MASLD and its complications, including deregulated iron metabolism. Further studies are underway to decipher the signaling pathway modulated by Suv420h1/h2 in hepatocytes.



Heme metabolism plays a critical role in sensitizing cells to ferroptosis

RUBAN AGARVAS Anand [1,2], LE TERTRE Marlène [1,2], FIORITO Veronica [3], MAO Lianghao [4], TOLOSANO Emanuela [3], JAYAVELU Ashok Kumar [4,5], MUCKENTHALER Martina [1,2,5]

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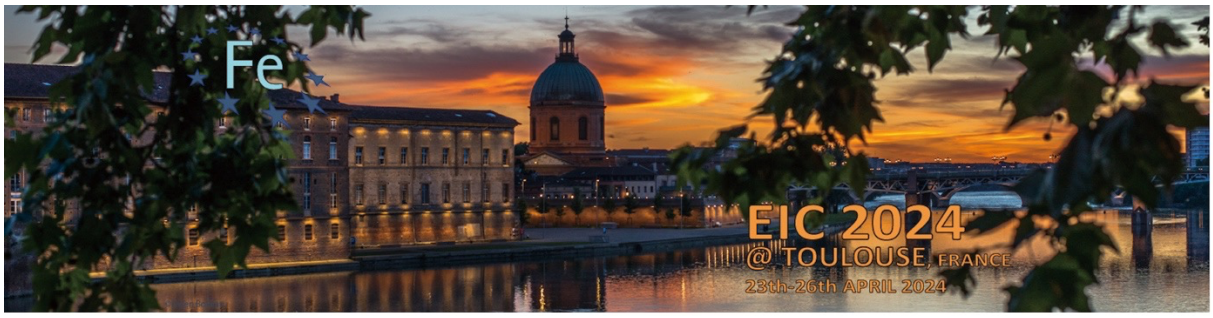
Iron plays a well-established role in ferroptosis, but the contribution of heme-bound iron remains unclear. Heme oxygenase 1 (HMOX1) mediated heme catabolism can release free iron, however, both pro-and anti-ferroptotic roles of HMOX1 have been reported. Here, we investigate how heme and its metabolism affect ferroptosis sensitivity. To induce ferroptosis, we deprived HT1080 cells of cysteine (either by switching to a cysteine-deficient medium or via treatment with Erastin, an inhibitor of cystine importer SLC7A11) and show that intracellular heme levels are increased 4h later. Both, inhibition of heme breakdown via HMOX1 inhibitors as well as inhibition of two enzymes (Aminolevulinic acid synthase 1 or Ferrochelatase) required for heme biosynthesis protected from ferroptosis. Interestingly, inhibition of heme synthesis further prevents the sensitization to ferroptosis by exogenous iron. These results strongly suggest that heme-bound iron plays an essential role in ferroptosis. To test the requirements for heme and iron in a time-dependent manner we induced ferroptosis and added either an HMOX1 inhibitor (Zinc Protoporphyrin IX), an iron chelator (Desferroxamine) or a ferroptosis inhibitor (Liproxstatin-1; lipid peroxide scavenger) at different time points. Intriguingly, we found that heme degradation is an early event that precedes the requirement for iron or lipid peroxidation during ferroptosis. Our results reveal important insights into how various iron sources influence ferroptosis and show that iron derived from heme is essential in the earliest phases of ferroptosis induction. Experiments are ongoing to understand the regulation of players of iron and heme metabolism during ferroptosis.



Ferroptosis causes pancreatic failure in a mouse model of non-HFE hemochromatosis (FpnC326S)

LE TERTRE Marlène [1], RUBAN AGARVAS Anand [1], CONRAD Marcus [2], BRABLETZ Thomas [3], BRABLETZ Simone [3], PENKOV Sider [4], FEDOROVA Maria [4], ALTAMURA Sandro [1], MUCKENTHALER Martina [1]

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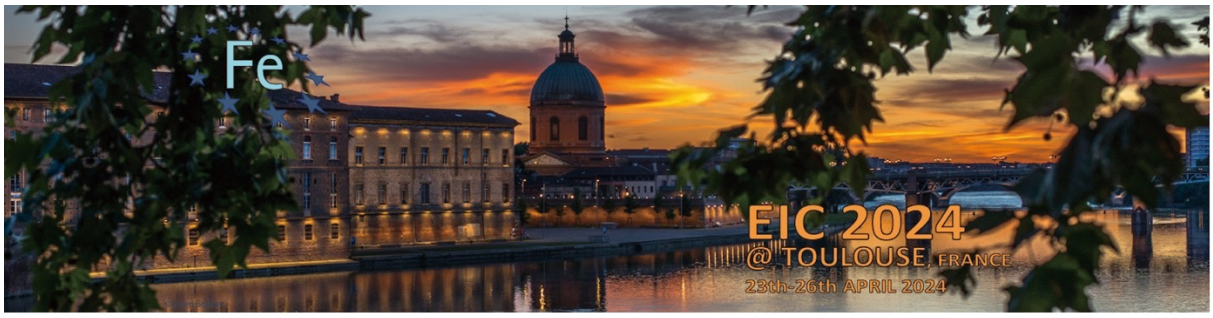
The non-HFE hemochromatosis Ferroportin(C326S) mice develop similar degrees of hepatic and pancreatic iron accumulation, associated with lipid peroxidation. However, in contrast to the liver, the pancreas is severely damaged by iron-overload, leading to exocrine pancreatic failure and early death. The underlying mechanisms and organ selectivity of tissue damage observed in these mice remain poorly understood. Of note, iron restriction prevents these complications, suggesting that ferroptosis explains exocrine pancreatic failure. We aimed to understand whether tissue iron-overload is sufficient to cause ferroptosis in FpnC326S mice and which mechanisms protect or sensitize tissues to ferroptosis. We demonstrate that the pancreas, unlike the liver, shows several ferroptosis hallmarks: accumulation of the lipid peroxidation product 4-HNE, ultrastructural mitochondrial changes, increased expression of the ferroptosis-related genes *Chac1* and *Acsl4*. RNAseq data suggest that iron overload in the pancreas is due to low ferroportin expression, while in the liver it is related to the high expression of NTBI importers. Additionally, iron overload may be more toxic in the pancreas as the organ is poorly equipped with antioxidant defences. Preliminary lipidomic analyses indicate organ-specific changes in lipid composition possibly contributing to the sensitivity/resistance to ferroptosis. To understand the chronology of the exocrine pancreatic failure, we performed histological analyses of tissues at different ages. We find that early pancreatic iron accumulation triggers lipid peroxidation. Consistent with the reported ferroptosis immunogenicity, we observe subsequent massive macrophage infiltration. Overall, our data are expected to discover novel therapeutic mechanisms that protect tissues from iron-mediated toxicity and ferroptosis in iron overload disorders.

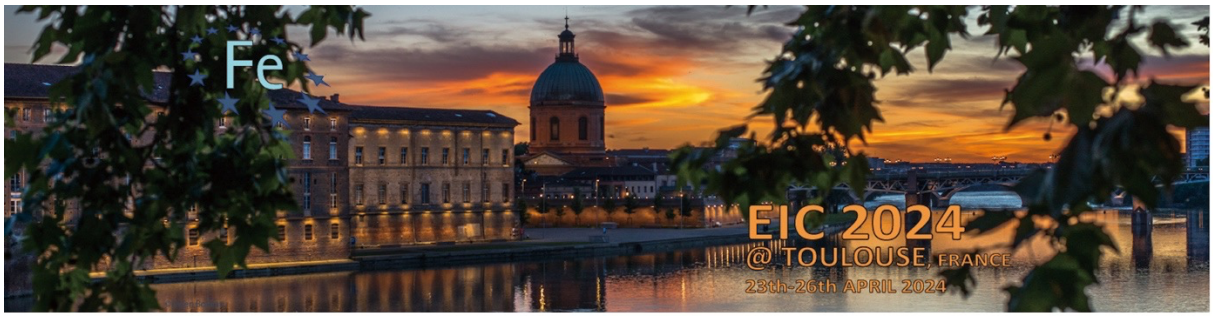


THE NOVEL ORAL SMALL MOLECULE CPD-348 CLOSELY MIMICS HEPCIDIN AND AMELIORATES ERYTHROCYTOSIS IN MICE

ALT Carsten [1], XU Qing [1], LIAOZOU Hilary [1], KAYA Emine [1], DEGUZMAN Francis [1], TRENKNER Elizabeth [1], LI Xiao-Jun [1], LEVINE Charles [1], CHEN Yu-Wei [1], IKEKHUA Sarah [1], HUANG Heli [1], CHANG Xiao [1], ZHANG Chenghong [1], LI Zhe [1], OKSENBERG Donna [1], CATHERS Brian E. [1]

[1] Pfizer, South San Francisco, United States

The 25 amino acid peptide hormone hepcidin regulates the systemic iron pool by inducing internalization and degradation of the cellular iron exporter ferroportin. Hepcidin replacement therapy could be applied to a variety of diseases including polycythemia vera (PV), hereditary hemochromatosis, β-thalassemia, and myelodysplastic syndrome. While multiple hepcidin mimetics have been developed, no orally bioavailable small molecule has been described that closely mimics hepcidin-mediated ferroportin internalization. In this present study we report the first oral small molecule hepcidin mimetic, Compound 348 (Cpd-348), that induces ferroportin internalization comparable to hepcidin, and ameliorates erythrocytosis in a mouse model of PV. Cpd-348 resulted in loss of luciferase activity in an inducible luciferase-tagged ferroportin internalization assay in CHO cells (IC50=10 nM). Furthermore, on T47D cells that endogenously express ferroportin, Cpd-348 diminished ferroportin surface expression with similar efficacy when compared to hepcidin whereas the previously published hepcidin mimetic vamifeport does not. Using a fluorescence polarization assay with recombinant ferroportin and labeled hepcidin, we observed that Cpd-348 directly inhibits the ferroportin–hepcidin interaction. Next, we tested the effect of Cpd-348 in vivo. In naïve mice, Cpd-348 mixed into the animal diet (0.03%, 0.1%, and 0.3%) caused dose-dependent microcytosis and reduction in reticulocyte hemoglobin. More interestingly, in a disease model of PV, Cpd-348 lowered serum iron concentrations and ameliorated the erythrocytosis. In summary, we report that Cpd-348 is the first oral small molecule hepcidin mimetic that closely mimics hepcidin function and ameliorates PV. Cpd-348 may be a therapeutic option for the treatment of PV and other hepcidin-related disorders.



THE NATURAL HISTORY OF FERROPORTIN DISEASE - RESULTS OF THE INTERNATIONAL, MULTICENTER EASL NON-HFE

ZOLLER Heinz [1,20], SCHAEFER Benedickt [2], **TROPPMAIR Maria [2]**, FIORINI Massimo [3], SCARLINI Stefania [3], PELUCCHI Sara [4], MARIANI Raffaella [5], PORTO Graça [6], BUSTI Fabiana [7], SANCHEZ Mayka [8], WEISSENSTEINER Hansi [9], SCHÖNHERR Sebastian [9], FORER Lukas [9], KRONENBERG Florian [9], PAMMER Lorenz M [2], KREMSER Christian [10], HENNINGER Benjamin [10], SANTOS Paulo C J L [11], PENG An [12], WANG Fudi [12], MARCO De Gobbi [13], UNAL Sule [14], NORIYUKI Yamakawa [15], DRAKESMITH Hal [16], TILG Herbert [2], BARDOU-JACQUET Edouard [17], GIRELLI Domenico [7], PIPERNO Alberto [18], PIETRANGELO Antonello [19], CORRADINI Elena [19]

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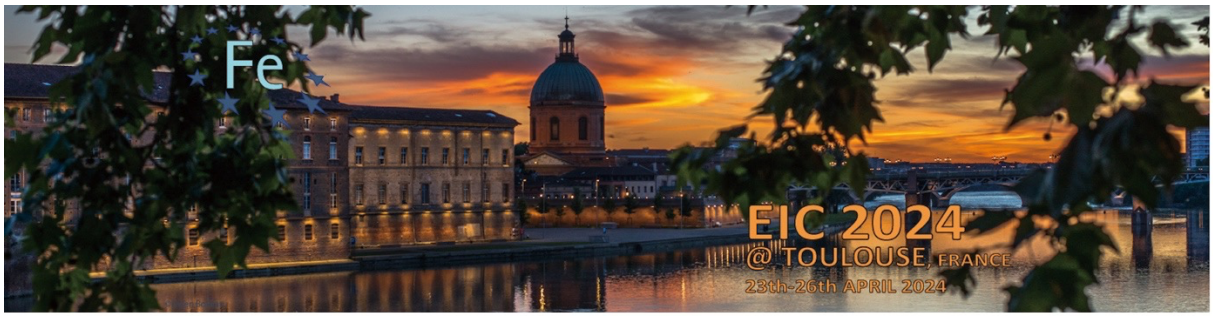
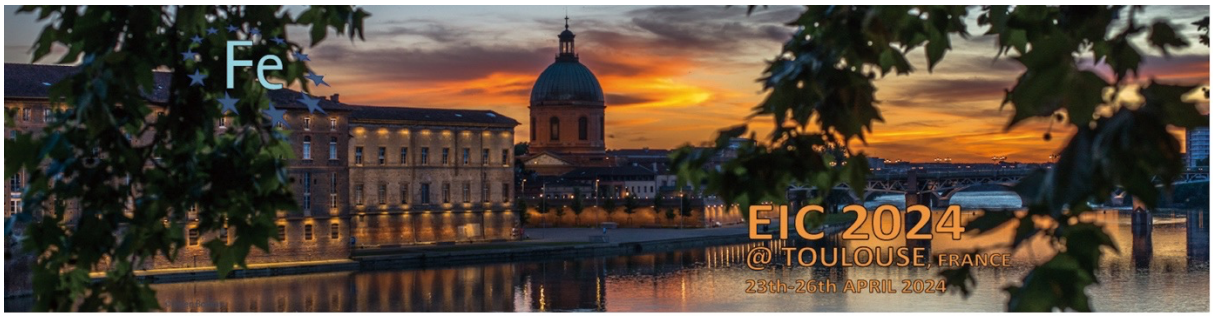
Background and Aims: Ferroportin (FPN) disease and SLC40A1-related hemochromatosis (former type 4B) are phenotypically distinct diseases caused by mutations in SLC40A1. Transferrin saturation and splenic/macrophage iron overload distinguish FPN disease from hemochromatosis. Prognosis and management of patients with SLC40A1 mutations has been inferred from HFE hemochromatosis, despite differences in phenotypic presentation. We aim to define the clinical characteristics and management of patients with SLC40A1mutations in comparison to HFE hemochromatosis.

Methods: The EASL non-HFE registry study collected structured clinical data from 95 patients with SLC40A1 mutations and was expanded by 339 published cases from a systematic literature search. Data were compared with an age and sex matched cohort of patients diagnosed with HFE C282Y homozygous hemochromatosis.



Results: Individuals with FPN disease had significantly lower serum iron, ferritin, and transferrin saturation when compared with hemochromatosis patients. Thirty-three percent of patients presented with a SLC40A1-related hemochromatosis phenotype. Median hepatic and splenic iron concentration was higher in individuals with FPN disease as compared to the HFE hemochromatosis group ($p<0.001$). Mean survival after diagnosis was 32.6 years in individuals with SLC40A1 mutations as compared to 24.4 years in HFEhemochromatosis patients ($p=0.509$). In the SLC40A1 group, 74% of patients received phlebotomies (median: 0.7/month) which was not associated with survival.

Conclusion: Higher ferritin and hepatic iron concentrations indicate more severe iron overload in patients with FPN disease compared to hemochromatosis patients, but both groups show comparable survival. In individuals with FPN disease treated with phlebotomy no survival benefit was observed.



HAEMOCHROMATOSIS HFE GENOTYPES AND CLINICAL PENETRANCE TO OUTCOMES: LESSONS FROM THE UK BIOBANK

ATKINS Janice [1], PILLING Luke C [1], LUCAS Mitchell R [1], BANFIELD Lucy R [2], KNAPP Karen [2], SHEARMAN Jeremy D [3], MELZER David [1]

[1] Epidemiology and Public Health Group, Department of Clinical and Biomedical Sciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom; [2] The Department of Health and Care Professions, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom; [3] Department of Gastroenterology, South Warwickshire University NHS Foundation Trust, Warwick, United Kingdom

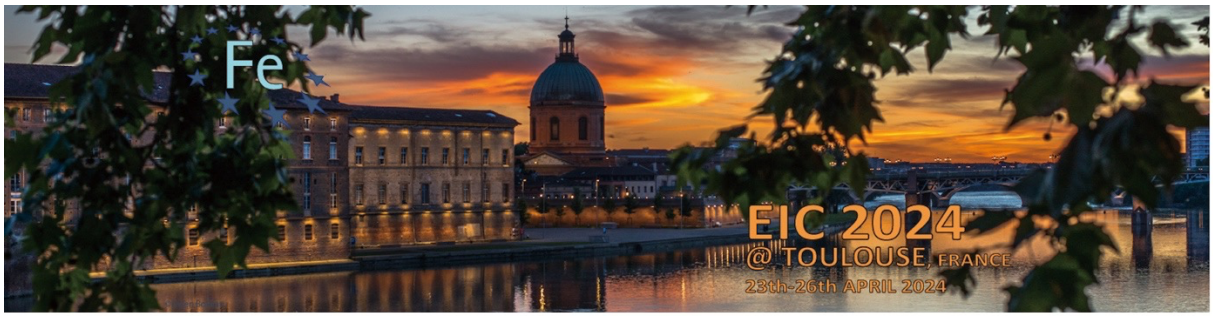
Background: In European ancestry populations, HFE genetic variants can cause iron overload and haemochromatosis, especially in p.C282Y homozygous (+/+) males. Clinical penetrance to disease in older ages is unclear. We used UK Biobank data to provide new life-course insights into morbidity within p.C282Y/p.H63D genotypes.

Methods:UK Biobank European genetic ancestry participants (n~451,000, 39-73 years) were followed-up for mean 13.3 years. Cox proportional hazards models assessed associations between HFE genotypes (p.C282Y/p.H63D) and incident outcomes. Analyses were stratified by sex and adjusted for age, genetic principal components, and assessment centre.

Results:2,902 participants were p.C282Y+/+ (0.6%). Male p.C282Y+/+ had increased risk of mortality (HR=1.29, 95% CI:1.12-1.48, p=4.7*10-4; cumulative incidence 33.1% by age 80 compared to 25.4% without HFE variants), liver disease (HR=2.56, 95% CI:2.10-3.12, p=8.70*10-21) and liver cancer (HR=7.90, 95% CI:5.46-11.43, p=5.50*10-28) compared to those without variants. Other non-HFE genetic variants also affected penetrance to liver disease/liver cancer. Male p.C282Y+/+ also had increased risk of prostate cancer (but not other cancers), joint replacement, Parkinson's disease, delirium, and dementia (HR=1.72, 95% CI:1.26-2.35, p=0.001; and higher brain iron deposition in magnetic resonance imaging), compared to those without variants.

Female p.C282Y+/+ had more modest excess morbidity, with no increased risk of menstrual problems, but increased risk of liver disease (HR=1.62, 95% CI:1.27-2.05, p=7.8*10-5) and joint replacement, especially post-menopause.

Conclusion: In this large community genotyped sample, male p.C282Y+/+, and to a lesser extent female p.C282Y+/+ was associated with substantial excess morbidity. There may be justification for targeted or community genotyping to identify haemochromatosis risks early in p.C282Y homozygotes



Transient Elastography, Fib4 and APRI for the diagnosis of liver fibrosis in HFE hemochromatosis

TRUBERT Lise [1], MASROUR Oumnia [1], MORCET Jeff [1], LAINE Fabrice [1], TURLIN Bruno [1], **BARDOU-JACQUET Edouard [1]**
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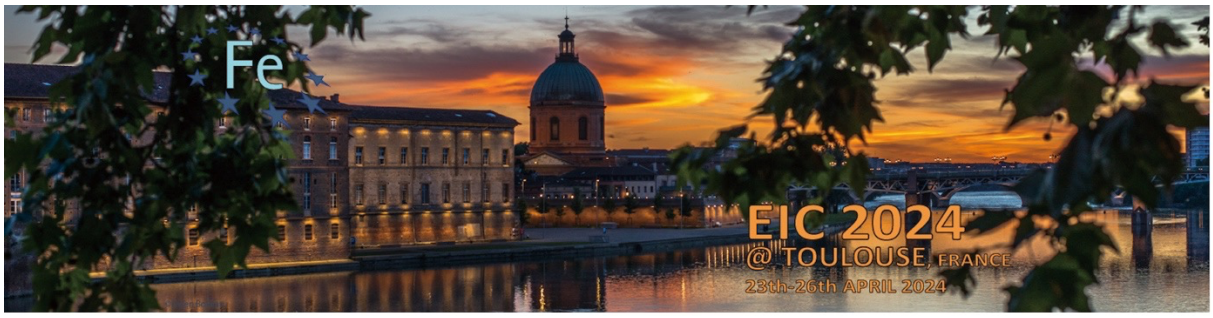
Background:
Current EASL guidelines recommend screening for liver fibrosis in all HFE hemochromatosis (HH) patients at diagnosis. Limited evidence exists comparing transient elastography (TE), Fib-4, and APRI to liver biopsy in this context. Our study aimed to evaluate their performance.

Method:
We included HH patients (2005-2023) who underwent both liver biopsy and TE within a year of diagnosis. Patients with TE>7.1kPa and IQR>0.3 were excluded. Diagnostic performance of TE, Fib-4, and APRI was assessed using ROC curves for severe liver fibrosis (Metavir F3F4).

Results:
A total of 127 patients (81.9% male, age 49.5±11.3) were included. Diabetes and excessive alcohol consumption were present in 10.2% and 7.9%, respectively. Ferritin levels were 1508µg/L±1321, and transferrin saturation was 85%±14.6. Fibrosis stage prevalence was F0F1=48.1%, F2=25.2%, F3=7.9%, and F4=18.9%. Mean TE was 9.8kPa±8.8. AUC for severe fibrosis diagnosis by ferritin, TE, Fib-4, and APRI were 0.92(0.86-0.96), 0.94(0.88-0.97), 0.85(0.77-0.91), and 0.83(0.75-0.90) respectively. Cutoff value with 90% sensitivity were ferritin:1853µg/L, TE:8.6kPa, Fib-4:1.18, and APRI:0.53. Cutoff value with 90% specificity were ferritin:2224µg/L, TE:9.9kPa, Fib-4:2.04, and APRI:0.87. Combining ferritin and TE criteria resulted in correct classification of 82(64%) patients, without false negatives/positives. However, 32(25%) had discrepant results, and 12(9%) were in the intermediate zone requiring liver biopsy.

Conclusion:
Our findings suggest that APRI and Fib-4 have lower performance compared to ferritin and TE in HH patients. We propose TE cutoffs of 8.6kPa to rule out severe fibrosis and 9.9kPa to confirm it. Overall, combining non-invasive tests significantly reduce the need for liver biopsy in HH patients.



HEMATOLOGICAL SEQUELAE OF HFE P.C282C HOMOZYGISITY IN FOUR LARGE GENETIC COHORTS

GEIRSDOTTIR Inga S. [1], **MAGNUSSON Magnus K [1]**
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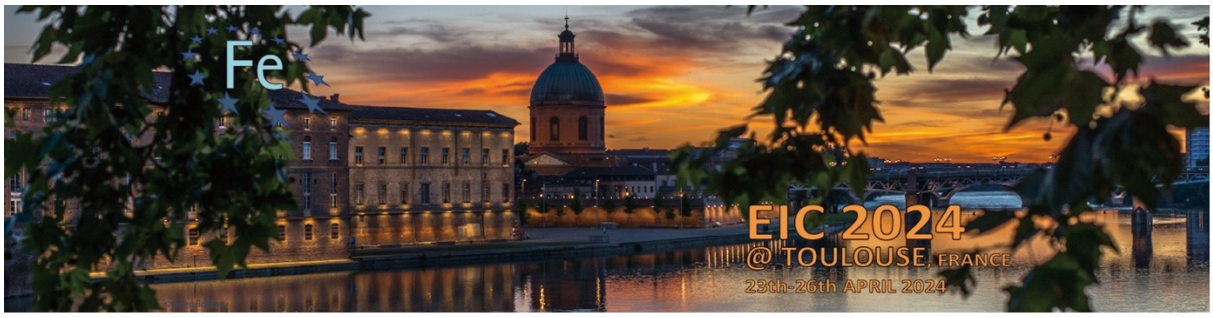
Genetic cohorts provide powerful tools to assess in an unbiased manner the longterm consequences of specific genotypes. HFE p.C282Y homozygosity is characterized by disordered systemic iron homeostasis leading to hereditary hemochromatosis in a subset of people. Recent studies have suggested that HFE p.C282Y homozygosity is overrepresented in polycythemia vera (PV) and disordered hepcidin suggested to govern the erythroid phenotype. We have performed a phenome wide association meta-analysis on four genetic cohorts (970,000 individuals, 5982 being HFE homozygous). The analysis included extensive case-control and continuous quantitative phenotypes. We present here the results for hematological phenotypes. The cohorts were from Iceland (deCODE genetics), the UK (UK Biobank), Denmark (Copenhagen Hospital Biobank/Danish Blood Donor Study) and USA (Intermountain Healthcare). Sub-cohorts within UK and Iceland included data on clonal hematopoiesis, including JAK2-V617F mutations.

C282Y homozygotes had a higher prevalence of PV (OR=7.24 $p=4.37x10^{-24}$), while no association was seen with clonal hematopoiesis (OR=1.09, 95% CI 0.88 to 1.34, p=0.43). Strong association was found between C282Y homozygosity and increased hemoglobin (0.41 SDs, $p=6.84x10^{-142}$), MCH (0.98 $p=<1x10^{-300}$) and MCV (0.82 $p=<1x10^{-300}$). RBC was decreased (-0.27 $p=1.06x10^{-50}$), while reticulocyte count (0.30, $p=1.42x10^{-54}$) and percentage (0.36 $p=7.91x10^{-79}$) were increased. C282Y homozygotes diagnosed with PV, unlike other PV cases lacked association with JAK2-V617F mutations and their hematological phenotyped matched the phenotype seen in non-PV C282Y homozygotes and were distinct from the hematological phenotype seen in PV. Homozygous individuals have increased risk of non-clonal primary polycythemia, while association with polycythemia vera is likely due to misdiagnosis exacerbated by increased hemoglobin levels.



Poster session | PS-01 - Poster session 1

P-01 - Laser Ablation coupled to ICP/MS provides multi-elemental resolutive spatial distribution in tissues

ALLAUME Pierre [1], LE MAITRE Johann [2,3], TURLIN Bruno [1,4], ISLAND Marie-Laure [2,3], LEROYER Patricia [3,4], SURGET Marie [3,4], BARDOU-JACQUET Edouard [4,5], ROPERT-BOUCHET Martine [2,3,4], **LORÉAL Olivier [3,4]**

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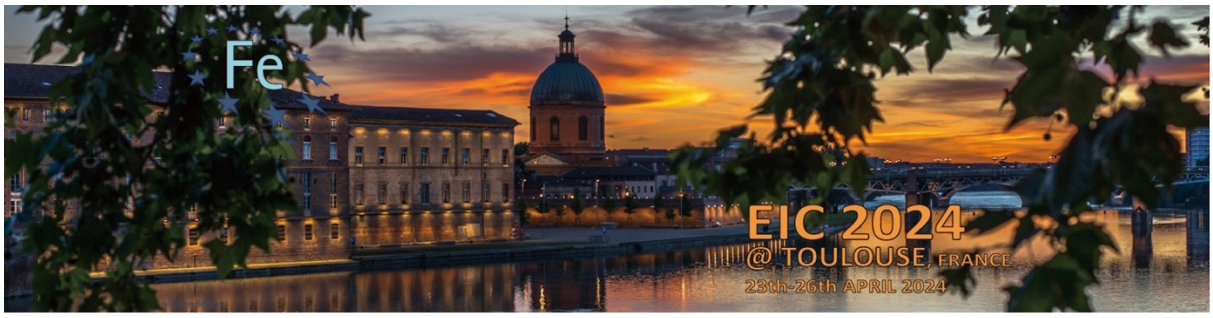
Background : Iron and other essential metals contribute to homeostasis. Metal metabolisms imbalance favors the appearance or progression of diseases. In tissues, metal concentration is measured biochemically as a whole, but this does not inform on the localization of metals. Specific stains for iron and copper can be used but their sensitivity is low. Being able to assess in tissue samples the parallel localization of these metals would help to evaluate their role the progression of diseases.

Aim : Our objective was to image metals in paraffin embedded human liver biopsies by using laser ablation-inductively coupled mass spectrometry (LA-ICP-MS) in order to evaluate the interest of the method. Methods: Liver samples were collected in Rennes Hospital, from patients exhibiting genetic hemochromatosis or Wilson's disease. In addition, the cerebellum of normal rats was investigated to evaluate the input of LA-ICP-MS on metal location in a architecturally heterogenous tissue. Fe, Cu, Zn, Se and Mg distributions were analyzed.

Results: LA-ICP-MS demonstrates local iron and copper distribution in the liver of patients, thus giving additional information to the concentration level. Distribution of other metals, unable to evaluate by histological staining, can also be evaluated. Local quantification of iron and copper can be performed. In the cerebellum, close to Purkinje cells line, iron and copper, not detected using classical staining, were found.

Conclusion: LA-ICP-MS is a highly sensitive and resolutive method for simultaneous location of metals in paraffin embedded samples. This method opens new opportunities for studying iron and metal homeostasis disorders.



P-02 - FATIGUE IN PATIENTS WITH HEREDITARY HEMOCHROMATOSIS: A VALIDATION OF FATIGUE ASSESSMENT TOOLS

SWIATCZAK Michal [1], RACZAK Alicja [2], SWIATCZAK Agata [3], MLODZINSKI Krzysztof [1], SIKORSKA Katarzyna [4], DANILOWICZ-SZYMANOWICZ Ludmila [1]

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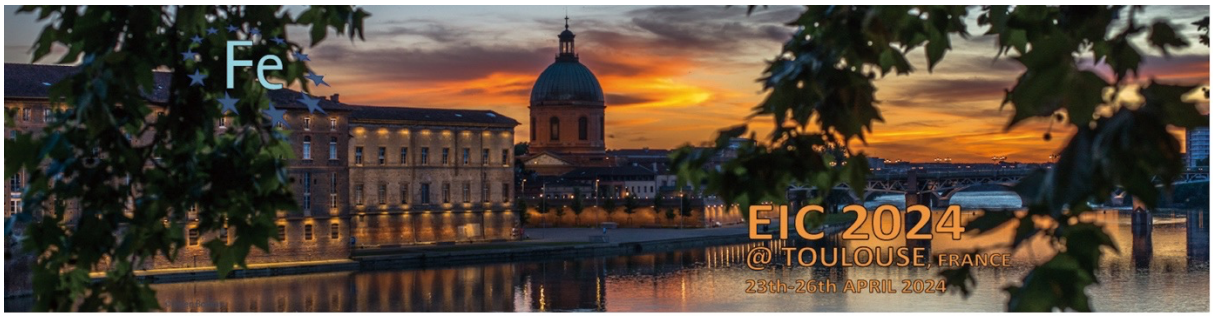
Background.
Hereditary hemochromatosis (HH) is a genetic condition with fatigue as one of its essential symptoms. There are some well-specified scales for fatigue assessment in other pathologies, but there is no data regarding their usefulness in HH. This research aimed to validate the Fatigue Assessment Scale (FAS), Fatigue Severity Scale (FSS), and Chalder Fatigue Scale (CFQ) and evaluate fatigue levels for HH patients.

Methodology.
A questionnaire containing items about detailed medical histories and all three fatigue assessment scales was administered to 79 HH patients, 25 healthy non-blood donors (CG), and 30 blood donors (BD). A standard procedure was used for the translation of all scales.

Results.
Excellent internal consistency and reliability for each scale were revealed in HH patients: Cronbach Alfa for FAS: 0.92, FSS: 0.95, CFQ: 0.93. Parameters obtained on three scales were significantly worse for patients with HH than in CG and BD (HH vs. CG p-value: FAS = 0.003, FSS = < 0.001, CFQ = 0,003; HH vs. BD p-value: FAS = 0.025, FSS = < 0.001, and CFQ = 0.041).

Conclusions.
That was the first implication of fatigue assessment scales in the HH population, which confirmed their excellent internal consistency and reliability. HH patients were characterized by significantly worse parameters on all scales, which indicates stronger fatigue. Our results demonstrate that FAS, FSS, and CFQ could be simple and reliable instruments to assess and quantify fatigue for clinical and research purposes in HH patients.



P-03 - GLYCOSYLATED FERRITIN: A BIOMARKER TO HELP IN THE DIAGNOSIS OF FTL VARIANTS?

COUETTE Aurélien [1], ZERROUKI Salim [1], HAMDI ROZE Houda [2,4], DETIVAUD Lénaïck [2], LOREAL Olivier [4], ISLAND Marie-Laure [1], BARDOU JACQUET Edouard [3,4], ROPERT-BOUCHET Martine [1,4]

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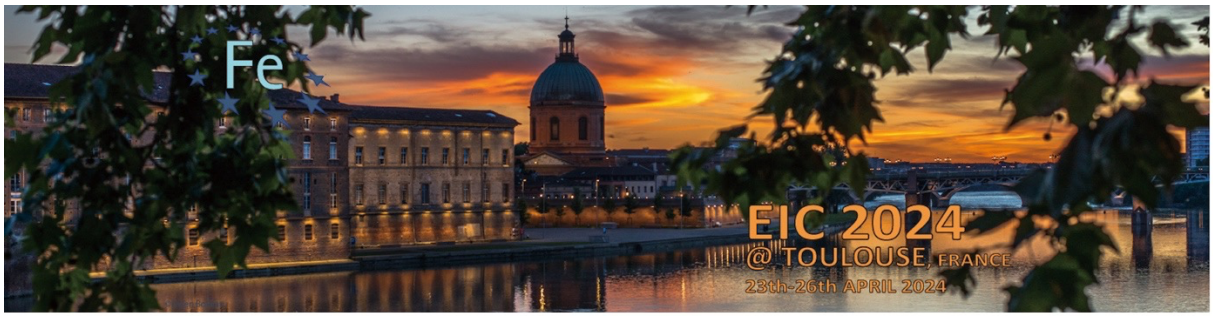
Background :
The diagnosis of hyperferritinaemia related to FTL variants (Exon 1, IRE 5' loop) is probably underdiagnosed. The aim of this study is to propose Glycosylated Ferritin (GlcFe) as a biochemical marker to aid in the diagnosis of unexplained hyperferritinaemia without iron overload.

Method:
Retrospective, observational, unicentric study since 2010 in a population of patients with hyperferritinaemia in whom GlcFe was measured by electrochemiluminescence after separation by ConcavalinA, compared with the clinico-biological and genetic diagnosis.

Results:
164 patients were included. In 21 Exon 1 patients, mean GlcFe was 98.9%±0.5 with mean total ferritin was 3811µg/l±3269. Mean GlcFe was 61.9%±7.4 in 25 IRE 5' loop patients with mean total ferritin was 1400µg/l ±772. In 73 patients with metabolic hepatosiderosis, mean GlcFe was 81.6%6±7.1 with mean total ferritin was 713µg/l±251. While reference values of GlcFe (67-88%) have been established for a healthy population (n=236), we also analysed 45 asymptomatic "control" patients with hyperferritinaemia and normal CRP in whom the mean GlcFe was 84.1%±4.7, and mean total ferritin was 457µg/L±180. We observed that 100% of patients diagnosed with an Exon 1 mutation had a GlcFe greater than 97%, while ferritin glycosylation was below 68% for 90% of patients diagnosed with IRE 5' loop mutation.

Conclusion:
The use of glycosylated ferritin would be a a simple and inexpensive first line biochemical test for the diagnosis of hyperferritinemia. A value greater than 97% or lower than 68%, this marker would suggest FTL sequencing with a high sensitivity and specificity.

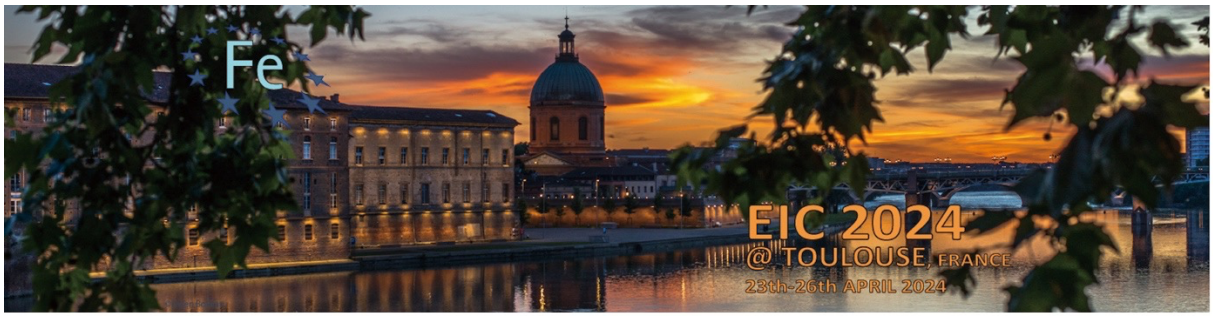


P-04 - Osteoporotic fracture risk is associated with higher plasma ferritin and serum iron level in females

ROBIN François [1,2], ROPERT-BOUCHET Martine [1,3], CADIOU Simon [2], ISLAND Marie-Laure [1,3], BERTHOUD Olivia [2], GUGGENBUHL Pascal [1,2], **LOREAL Olivier [1]**

[1] Univ Rennes, INSERM, Rennes University Hospital, UMR 1317, Institut NuMeCan (Nutrition Metabolisms and Cancer), Rennes, France. , Rennes, France; [2] Rheumatology Department, University Hospital, Rennes, France., Rennes, France; [3] Medical Biochemistry Department, University Hospital, Rennes, France., Rennes, France

Knowing that iron overload has been associated to osteoporosis, we investigated whether plasma metals concentrations, including iron parameters, were associated to the occurrence of fractures in osteoporotic patients. Two patients' groups were included in a prospective monocentric study: Patients with severe osteoporotic fracture (n=125; OF) and a control group with abnormally low bone mineral density (BMD) without fracture (n= 100; OD). Routine blood tests were performed to explore bone diseases. ICP-MS was used to measure plasma concentrations of metals of interest (iron, copper, zinc, selenium, strontium, molybdenum, manganese, cobalt, cadmium, lead and aluminum). BMD was determined using the same machine. Fractured patients were older (77±11 vs 66±13 years, $p<0.001$). There was no significant difference between the two groups for gender (80% female vs. 72% for OF and OD respectively), blood calcium, phosphorus, 25OH-vitamin D, CRP and parathyroid hormone. In overall population, ferritin was significantly higher in the OF group than in the OD group (273±276 vs 199±227 ng/ml ($p<0.05$)). The difference was related to the female population (239±232 vs 164±181ng/ml ($p<0.05$). In OF women, serum iron and selenium levels were lower ($p<0.05$). In OF men, selenium level was decreased ($p<0.05$) and aluminum concentration was increased ($p<0.05$). Our results show in women an association between higher ferritin and serum iron and the fracture risk. Increased aluminum appears to be associated with an increased risk of fracture in men. Decreased serum selenium appears to be associated of fracture risk in both sexes.



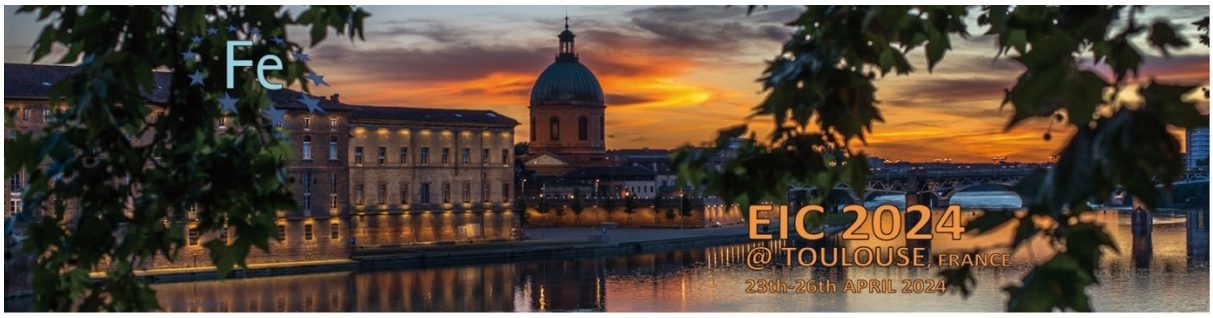
P-05 - iron signature in pulmonary hypertension associated with chronic obstructive pulmonary disease

MYRONENKO Oleh [1], FORIS Vasile [1,3], CURCIC Pero [2], OLSCHEWSKI Andrea [3,4], OLSCHEWSKI Horst [1,3]
[1] Division of Pulmonology, Medical University of Graz, Graz, Austria; [2] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; [3] Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria; [4] Department of Anaesthesiology and Intensive Care Medicine, Medical University of Graz, Graz, Austria

Introduction. Chronic obstructive pulmonary disease (COPD) is a leading cause of high morbidity and mortality worldwide. Pulmonary hypertension (PH) is prevalent in the majority of COPD patients and can lead to right heart failure with a very poor prognosis. While COPD lungs exhibit a higher iron content in the epithelium and alveolar macrophages (AMs) compared to healthy controls, the mechanisms behind local iron overload and involvement of the pulmonary vasculature in this process are not yet clear. Moreover, up to 50% of COPD patients develop systemic iron deficiency associated with more severe PH, and *IREB2* has been identified as a COPD susceptibilty gene. Conflicting results exist regarding the impact of iron on the development of experimental COPD and PH in animal models.

Objectives. We aimed to characterize circulating iron-associted factors, gender- and lung compartment-specific iron distribution, as well as presence of an "iron signature" in the transcriptomic profile of severe PH in COPD lungs.

Results. High levels of circulating soluble transferrin receptor-1 were associated with severe PH in COPD, corresponding to the levels of transferrin receptor in the lungs. Ferric iron-loaded cells in lung sections were negatively correlated with NT-proBNP and were significantly enriched in COPD patients with moderate PH, but not severe PH. Distinctive patterns of iron distribution in COPD lungs with severe PH were identified. Transcriptomic profiling of bronchi revealed down-regulated ferrous iron binding (GO “Molecular function”), and upregulated pulmonary artery morphogenesis (GO “Biological process”) signatures in COPD with severe PH compared to controls.



P-06 - FUNCTIONAL TEST TO ASSESS THE IMPACT OF VARIANTS IDENTIFIED IN THE 3'UTR REGION

DETIVAUD Lénaick [1,2,5,6], BOUVET Régis [1,6], ROPERT Martine [2,3], LALAND Mathilde [2,5], LOREAL Olivier [2,4], BARDOU-JACQUET Edouard [2,4,5], HAMDI-ROZE Houda [1,2,4]

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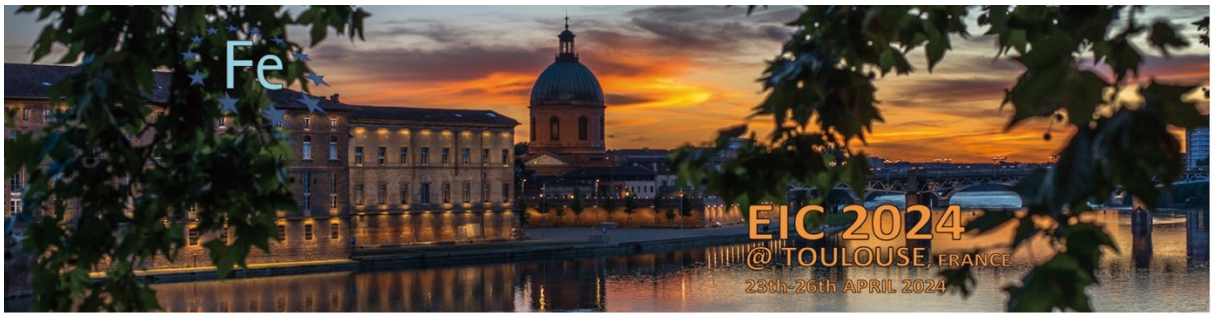
In the past few years, patients harbouring iron metabolism disorders have been undergoing high throughput sequencing. These technologies generate an important number of variants, complexifying their classification, especially when identified in non-coding sequences. 5' and 3' untranslated regions (UTRs) are involved in mRNA post-transcriptional regulation. UTRs are the target of different factors (proteins, ribonucleoproteins, LcRNA, mir RNA,...), that recognise specific nucleotides sequences or structures. Variants in these regions can affect transcript expression, stabilisation or degradation, which may lead to the translation of an incorrect protein expression. In this study, we propose a functional test to study the impact of 3'UTR variations. 3'UTR sequences are often associated with transcript stability or degradation. Wild type and mutated 3'UTRs are cloned in a luciferase reporter vector. The secreted luciferase depends on the 3'UTR and can be measure by a chemiluminescence assay. A SEAP gene is included in the vector and serves as a transfection normalizer in appropriate cell lines.

This model has been tested on the SLC11A2 3'UTR, using a variant identified in our patient cohort and a variant induced by site-directed mutagenesis in the IRE loop.

We thus provide proof of concept for this model to validate variants identified in 3'UTR

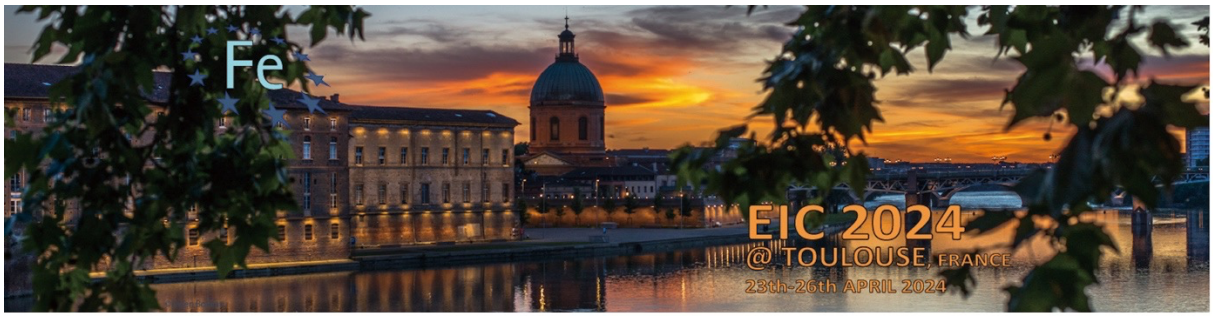


P-07 - ADDITIVE EFFECT OF MULTIPLE GENETIC VARIANTS IN SEC23B-PIEZO1 ON IRON METABOLISM DYSHOMEOSTASIS

NOSTROSO Antonella [1,2], ROSATO Barbara Eleni [1,2], MARRA Roberta [1,2], ESPOSITO Federica Maria [1,2], D'ONOFRIO Vanessa [1,2], RIBERSANI Michela [3], BULLA Anna [4], DEL VECCHIO Giovanni Carlo [5], MANDRILE Giorgia [6], CEGLIE Teresa [6], IOLASCON Achille [1,2], ANDOLFO Immacolata [1,2], RUSSO Roberta [1,2]

[1] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli 'Federico II', Napoli, Italy; [2] CEINGE Biotecnologie Avanzate Franco Salvatore, Napoli, Italy; [3] Università Sapienza di Roma Sezione Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Roma, Italy; [4] Azienda Ospedaliera Universitaria Policlinico "G. Rodolico – San Marco" , Catania, Italy; [5] U.O.C. Pediatria Generale e Specialistica " Bruno Trambusti", Bari, Italy; [6] SSD Microcitemie, AOU San Luigi Gonzaga, Regione Gonzole 10, Orbassano, Italy

Hereditary anemias (HA) are heterogeneous conditions characterized by a complex genotype-phenotype correlation. The two main groups include hypo-productive anemias and hemolytic anemias due to altered deformability of erythrocytes. Within these groups, the most frequent conditions are, respectively, congenital dyserythropoietic anemia type II (CDAII) (caused by biallelic mutations in the *SEC23B*) and dehydrated stomatocytosis (DHS) (mostly caused by gain-of-function mutations in the *PIEZO1*). Although the different pathogenesis, iron overload, and reduced hepcidin levels represent their main complications. Hereditary anemias can be caused by multi-locus inheritance. Indeed, in our cohort of patients (n=583), 34% exhibit multi-locus inheritance. Among them, 8 patients exhibit pathogenic variants in both *SEC23B* and *PIEZO1* genes. They show intermediate levels of anemia (RBC 3.7x106/µL, Hb 10.1 g/dL, MCV 92.3 fL) but a more pronounced iron overload (ferritin 646.3 ng/mL, IS 83.4%) compared to patients with isolated defects. *In vitro* functional assays demonstrated that silencing of *SEC23B* in a cellular hepatic model (Hep3B) engineered with R2456H-*PIEZO1*-GoF variant (Hep3B-KI) induced a stronger downregulation of *HAMP* gene expression compared to Hep3B-WT, Hep3B-shSEC23B, and Hep3B-KI. Additionally, shSEC23B/PIEZO1-KI showed increased ferritin expression at both mRNA and protein levels. To further characterize the alteration of iron status in shSEC23B/PIEZO1-KI, we assessed the inhibition of the BMP6/SMADs pathway. Analysis of target genes revealed a more pronounced downregulation of *SMAD6*, *ID1*, and *ID3* in shSEC23B/PIEZO1-KI, compared to Hep3B-shSEC23B and Hep3B-KI. These data confirm the additive effect of the two causative genes on iron dyshomeostasis and the importance of accurate diagnostic classification for personalized medicine.



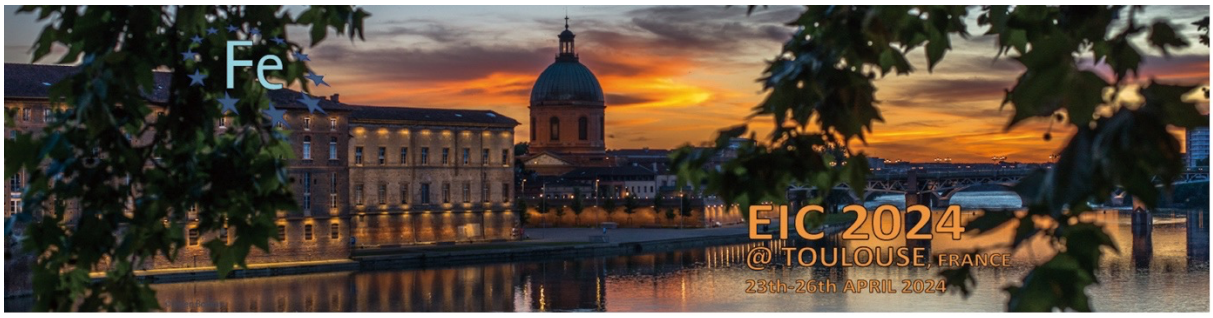
P-08 - CONTRIBUTION OF FUNCTIONAL TESTS TO THE STUDY OF SPLICE VARIANTS IN IRON METABOLISM DISORDERS

DETIVAUD Lénaick [1,2,3,6], BOUVET Regis [1,3], ROPERT Martine [2,4], CARRE Wilfrid [1,3], LALAND Mathilde [2,6], LOREAL Olivier [2,5], BARDOU-JACQUET Edouard [2,5,6], HAMDI-ROZE Houda [1,2,5]

[1] Laboratoire de Génétique Moléculaire et Génomique, Hôpital Pontchaillou CHU Rennes, 2 rue Henri Le Guilloux 35000, Rennes, France; [2] National Reference Center for Hemochromatosis and iron metabolism disorder, CHU Rennes, F-35000, Rennes, France; [3] CNRS, Institut de génétique et développement de Rennes, Université de Rennes - UMR 6290,, Rennes, France; [4] Laboratoire de Biochimie-Toxicologie, Hôpital Pontchaillou CHU Rennes, 2 rue Henri Le Guilloux, 35000, Rennes, France; [5] Univ Rennes, INSERM, INRA, Institut NuMeCan, CHU Pontchaillou – 2 rue Henri Le Guilloux – 35033 Cedex, Rennes, France; [6] Service des maladies du foie, Hôpital Pontchaillou CHU Rennes, 2 rue Henri Le Guilloux, 35000, Rennes, France

Various mutations in coding sequences have been documented in iron metabolism disorders. High throughput DNA sequencing data reveal a growing number of variations in non-coding regions. The impact of these variations need to be assessed in order to guide interpretation and classify the mutation. In this study, we focus on splicing variants identified in our cohort of nation-wide recruited patients harbouring iron metabolism disorders. The splicing process occurs during RNA maturation and is finely regulated by multi-factor complexes that interact with specific nucleotide domains, depending on tissue specificity, environmental or developmental signals. Variations affecting these sequences may induce non-appropriate alternative splicing and thus lead to a loss of function or expression of the protein. These variants are increasingly studied in all pathologies, preferably by analysis of transcripts on blood samples of patients.

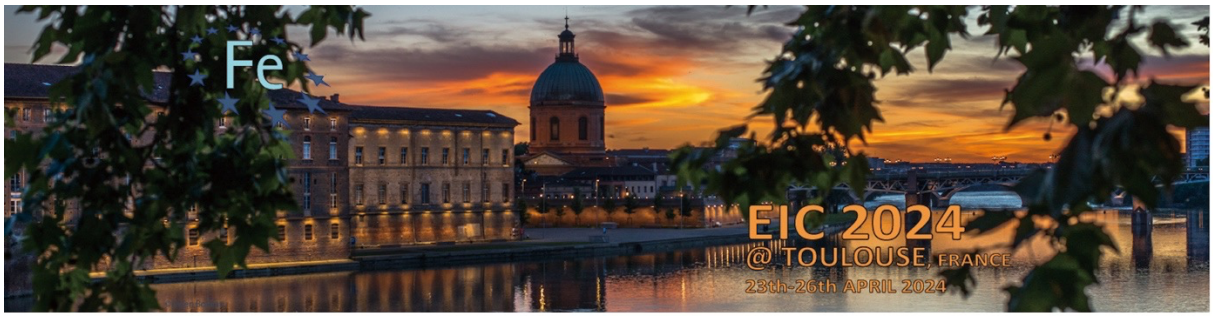
Variants selected for this study were predicted to affect splicing process in different genes of iron metabolism (TFR2, HFE, SLCA11A2, TF and CP). Minigene assay and, when available, patients' blood transcripts analysis (RNA targeted PCR and/or RNA sequencing), were performed to determine the impact of these variants. Experimental results were compared with patients' clinical data. This study showed us that even if these assay can converge and allow to classify certain variants, others present a discrepancy of results depending on the test used. Moreover, due to the low blood expression of these genes, minigene functional test in different cell lines could be more helpful to classify splicing variants in iron metabolism disorders.



P-09 - TMPRSS6 NON-CODING VARIANTS IN IRIDA PHENOTYPE EXPRESSION IN MONOALLELIC AFFECTED INDIVIDUALS

HOVING Vera [1], DONKER Albertine [4], SMEETS Roel [5], VAN DEN HEUVEL Bert [5], SCHOLS Saskia [1], SWINKELS Dorine [2,3]
[1] Department of Hematology, Radboud university medical center, Nijmegen, Netherlands; [2] Radboud Laboratory for Diagnostics, Department of Laboratory Medicine, Radboud university medical center, Nijmegen, Netherlands; [3] Sanquin Blood Bank, Amsterdam, Netherlands; [4] Department of Paediatrics, Maxima Medical Center, Veldhoven, Netherlands; [5] Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands

Iron refractory iron deficiency anemia (IRIDA), a rare inherited disorder, arises from pathogenic variants in the TMPRSS6-gene. It presents as microcytic anemia with low serum iron concentration and inappropriately high hepcidin levels. Although generally considered an autosomal recessive disorder, some patients express the phenotype with a monoallelic pathogenic exonic TMPRSS6-variant. The underlying pathophysiology in these patients remains unknown, causing diagnostic uncertainty. This retrospective monocenter study explored non-coding variants as potential contributors to the IRIDA-phenotype through full-sequencing of TMPRSS6. Symptomatic carriers (monoallelic IRIDA-genotype with phenotype), asymptomatic carriers (monoallelic IRIDA-genotype without phenotype expression) and wild-type relatives (no TMPRSS6 variant, no phenotype) were included. Whole-exome sequencing of iron-regulating genes was performed to exclude polygenic inheritance. Data were analyzed at family level and at individual level for subjects with and without included relatives, respectively. Variants were considered contributors if they were i) deemed potentially pathogenic based on in silico tools, and for those found in families ii) inherited in trans.Polygenic inheritance was excluded. Full-sequencing of TMPRSS6 in 27 subjects revealed 224 non-coding variants. Familial segregation analysis of these variants identified 13 trans-inherited variants, including one potentially pathogenic according to in silico tools. Individual-level analysis revealed that 21 of these 224 were found exclusively in symptomatic carriers without included relatives, with three potentially affecting splice sites and one located in the predicted promoter region. No variant was found exclusively in symptomatic carriers. Full-sequencing of TMPRSS6 revealed non-coding variants potentially influencing phenotypic expression. Further functional studies are warranted to confirm their implications for patient care.



P-10 - IRON OVERLOAD INDUCES GLUCOCORTICOID RESISTANCE IN MICE

PAGANONI Rossana [1], KNOOP Paul [1], STEELE-PERKINS Peter [1], VUJIC SPASIC Maja [1]
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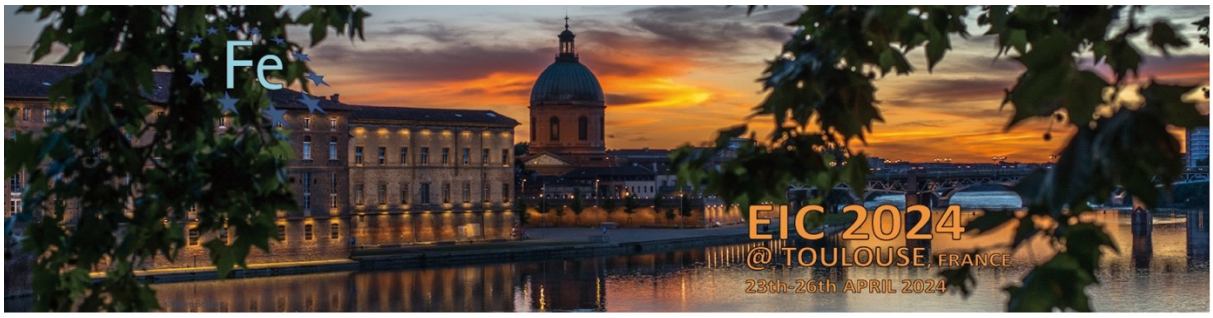
Secondary iron overload (IO) is a pathological condition related to chronic disease (thalassemia, sickle cell disease) and multiple transfusions. It is associated with significant impairment in the endocrine system, including diabetes and dysregulation of the hypothalamus-pituitary-adrenal/gonad axis. Glucocorticoids are the end targets of the HPA axis and regulate the immune response and cellular metabolism through the activation of the glucocorticoid receptor (Gr). Here, we investigate the effect of secondary iron overload on glucocorticoid metabolism and its signaling through Gr.

Mice were subjected to chronic IO through Iron-dextran (1g/kg) injections once per week for 8 weeks, or to acute IO with 24h treatment. Subsequently, Gr activity was measured in the liver and spleen.

We show that both treatments were sufficient to induce systemic iron overload, with a profound iron deposition detected in the liver and spleen. This was correlated with a significant decrease in total Gr protein levels and its phosphorylated forms (pGrSer211/203). We further demonstrate that liver non-parenchymal cells and the splenic F4/80 positive cells are target cells with reduced Gr signaling activity. Interestingly, the endocrine status of the HPA axis was unaffected implicating the tissue-specific glucocorticoid resistance mediated by iron excess. Indeed, exogenous GC administration, both *in vitro* and *in vivo*, was not sufficient to activate Gr signaling in target cells. In contrast, reducing iron-mediated oxidative stress (by N-acetylcysteine or Ferrostatin-1) restored cellular responsiveness.

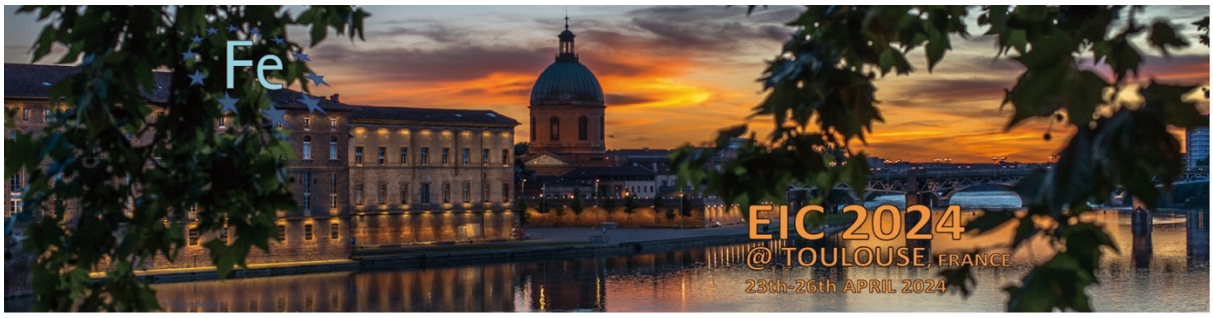
Our findings imply that iron retention in macrophages is a driver of glucocorticoid resistance *in vivo*, a condition that could be rescued by antioxidant drugs.



P-11 - BONE PHENOTYPING OF MURINE HEMOCHROMATOSIS MODELS WITH DEFICIENCIES OF HJV-, ALK2 OR ALK3

DOGAN Deniz Yildirim [1], HORNUNG Isabelle [1], PETTINATO Mariateresa [2], PAGANI Alessia [2], BASCHANT Ulrike [3], SEEBOHM Guiscard [4], HOFBAUER Lorenz C [3], SILVESTRI Laura [2,6], RAUNER Martina [3], STEINBICKER Andrea U [1]
[1] Department of Anesthesia, Intensive Care and Pain Medicine, Goethe University Frankfurt, Frankfurt am Main, Germany; [2] Regulation of Iron Metabolism Unit, Division of Genetics and Cell Biology,, IRCCS San Raffaele Scientific Institute, Milan, Italy; [3] Department of Medicine III & Center for Healthy Aging, Technische Universität Dresden,, Dresden, Germany; [4] IfGH–Cellular Electrophysiology, Department of Cardiology and Angiology, University Hospital of Münster,, Münster, Germany; [5] Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; [6] School of Medicine, Vita-Salute San Raffaele University, Milan, Italy

Osteopenia is observed in patients with iron overload, especially in HFE-dependent hereditary hemochromatosis (HH). While some mouse models of Hfe-HH show bone loss, this phenotype has not always been confirmed. Here, we assessed the bone phenotype of additional mouse models of iron overload, $Hjv^{-/-}$ mice and hepatocyte-specific Alk2 or Alk3 deficient mice to clarify under which circumstances high iron levels lead to bone loss. Bone phenotypes of 12-week-old mice with a global deletion of Hjv and hepatocyte-specific Alk2 and Alk3 deficient mice were investigated. Male $Alk2^{fl/fl}$;Alb-Cre mice were additionally raised on an iron-deficient diet to investigate the role of iron overload. Bone microarchitecture was examined using µCT. Bone remodeling was assessed using histomorphometry and serum bone turnover markers. Male and female $Hjv^{-/-}$ mice and female $Alk2^{fl/fl}$;Alb-Cre and $Alk3^{fl/fl}$;Alb-Cre had no altered trabecular or cortical bone mass or bone turnover, despite iron overload. Male $Alk2^{fl/fl}$;Alb-Cre and $Alk3^{fl/fl}$;Alb-Cre mice also presented with a regular trabecular bone mass at all ages, albeit 6-month-old $Alk3^{fl/fl}$;Alb-Cre mice showed an increased number of osteoclasts (+30%) and a lower bone formation rate (-50%). Cortical thickness of the femur was reduced in 6-month-old male $Alk2^{fl/fl}$;Alb-Cre and $Alk3^{fl/fl}$;Alb-Cre mice. Raising $Alk2^{fl/fl}$;Alb-Cre mice on an iron-deficient diet rescued the cortical bone phenotype. Cortical bone was affected in male $Alk2^{fl/fl}$;Alb-Cre and $Alk3^{fl/fl}$;Alb-Cre mice, suggesting specific roles for Alk2 and Alk3 in iron regulation of cortical bone that may be sex-specific. Whether the lack of trabecular bone loss is related to the relative hepcidin deficiency in these models remains to be investigated.



P-12 - Ceruloplasmin and Ferroportin interaction in macrophage iron efflux

PANZER Marlene [1,2], SCHAEFER Benedikt [1], WAGNER Sonja [1,2], PERTLER Elke [1,2], TROPPMAIR Maria [1], OBHOLZER Laura [1], PETZER Verena [3], WEISS Guenter [4], TALASZ Heribert [5], TILG Herbert [1], ZOLLER Heinz [1,2]

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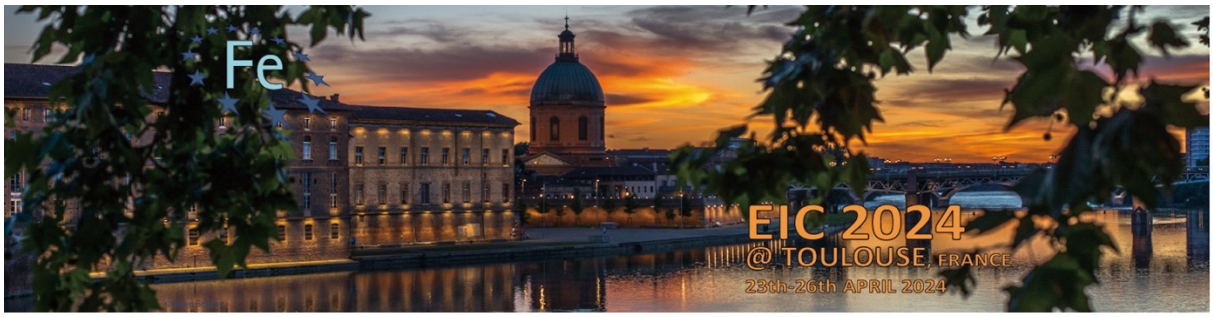
Macrophages are crucial for iron recycling from senescent red blood cells. The ferroxidase Ceruloplasmin (Cp) is an abundant protein in plasma and also implicated in iron export mainly from hepatocytes. In humans, recessive CP mutations cause aceruloplasminemia with iron overload in the liver and brain, but not the spleen. This study aims to investigate the function of ceruloplasmin in macrophage iron export, erythropoiesis and iron homeostasis.

Ferroportin-floxed mice with a macrophage-specific LysM-Cre recombinase (FPNDM/DM) were crossed with ceruloplasmin knockout (CP-/-) mice to generate CP-/-FPNDM/DM, FPNDM/DM, CP-/- and FPNflox/flox mice as controls. The impact on iron homeostasis and erythropoiesis was analyzed in 2 and 9-month-old female mice. Tissue iron, mRNA expression, blood count, red cell indices and plasma iron parameters were assessed. Erythropoiesis was analyzed by flow cytometry of the bone marrow.

Hemoglobin was significantly reduced in CP-/-FPNDM/DM and FPNDM/DM mice at 2 and 9 month, but only in 9 month old CP-/- mice. Plasma ferritin was elevated in CP-/-FPNDM/DM and FPNDM/DM and hepcidin-to-ferritin ratio was decreased. Transferrin saturation was reduced in all groups at 9 month. Iron was increased in the spleen of FPNDM/DM and CP-/-FPNDM/DM mice, whereas CP-/- and CP-/-FPNDM/DM mice had increased liver iron concentrations. In the bone marrow of CP-/- mice, iron was decreased at 9 months of age.

In conclusion, our findings suggest that ceruloplasmin is important for iron export from hepatocytes, whereas ferroportin is required for iron egress from macrophages. The phenotype of double knock out mice suggests limited functional interaction of ceruloplasmin and ferroportin.

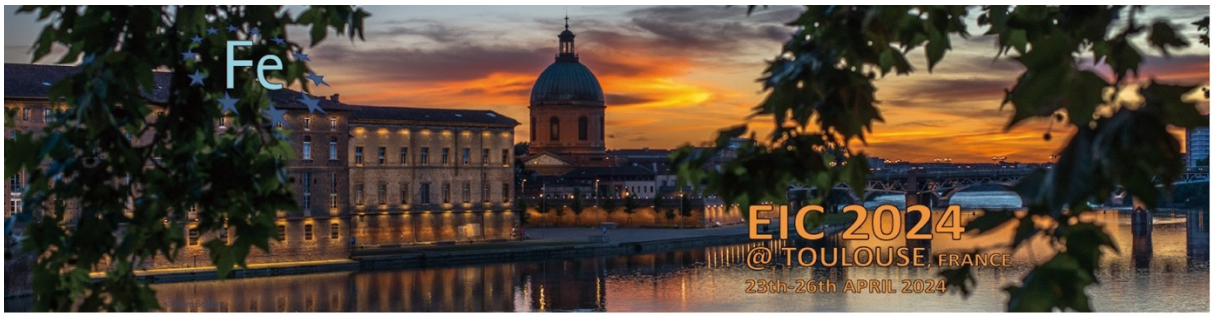


P-13 - PROTEOMICS AS A SENSITIVE TOOL TO INVESTIGATE IRON HOMEOSTASIS REGULATING MECHANISMS

KASTL Philipp [1], HELM Barbara [1], GÖDTEL Franziska [1], DIETER Yannik [1], QIU Ruiyue [2], STEELE-PERKINS Peter [6], VUJIC SPASIC Maja [6], MUCKENTHALER Martina U. [2,3,4,5], SCHILLING Marcel [1], KLINGMÜLLER Ursula [1,5]

[1] German Cancer Research Center (DKFZ), Heidelberg, Germany; [2] Department of Pediatric Hematology, Oncology and Immunology, Heidelberg University, Heidelberg, Germany; [3] Molecular Medicine Partnership Unit, University of Heidelberg, Heidelberg, Germany; [4] German Centre for Cardiovascular Research (DZHK), Partner site Heidelberg/Mannheim, Heidelberg, Germany; [5] Translational Lung Research Center Heidelberg (TLRC), German Center for Lung Research (DZL), University of Heidelberg, Heidelberg, Germany; [6] Institute of Comparative Molecular Endocrinology, Ulm University, Ulm, Germany

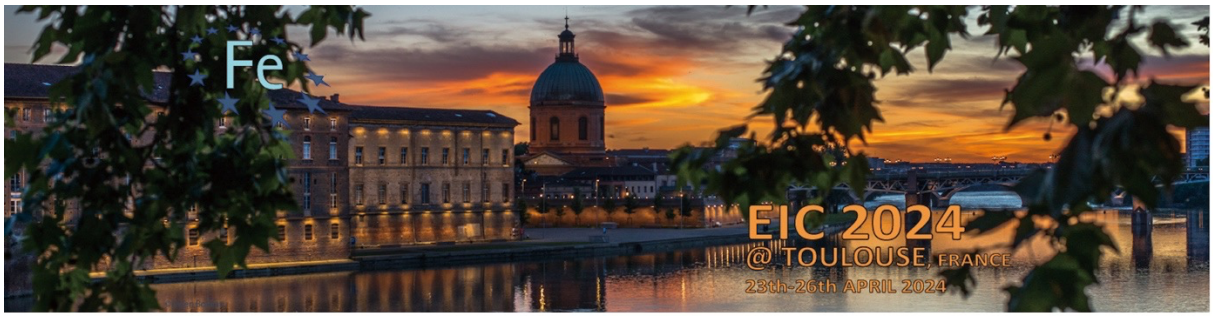
Iron homeostasis is tightly regulated and altered iron levels are associated with anemia, organ damage by iron overload and iron-induced ferroptosis. Iron is mainly stored in hepatocytes and liver and spleen macrophages, where its release is regulated by the hormone hepcidin. Hepcidin expression is mainly regulated by binding of BMP ligands to their respective receptor complex on hepatocytes. Even though numerous proteins involved in the regulation of iron homeostasis have been identified and their function established, a system-wide molecular understanding is missing. We developed a mass spectrometry-based proteomics pipeline comprising optimized sample lysis, automated sample processing, mass spectrometry-based data-independent acquisition and user-friendly bioinformatic analysis to elucidate iron-induced changes on the whole proteome level of primary murine hepatocytes treated with FeNtA overnight and blood plasma from wildtype mice injected with iron-dextran (0.1g/kg) for 8 weeks. We reliably identified and quantified proteins involved in iron homeostasis in primary mouse hepatocytes such as BMP pathway components, including BMP receptors. In iron-treated hepatocytes our data showed an increase in ferritin and a reduction of serotransferrin and transferrin receptor 1, as well as an increase of ferroptosis-associated factors including Gluthatione S-transferase P1 (Gstp1). Interestingly, we also observed an increase in ferritin and Gstp1 and a reduction of serotransferrin/transferrin receptor 1 in the plasma of iron-injected wildtype mice. Additionally, other hepatic enzymes involved e.g. in energy metabolism, like Sorbitol dehydrogenase or Aldolase B, were increased in the plasma of these mice indicating iron-induced liver damage and thus could serve as novel markers of iron overloading conditions.



P-14 - CHANGES IN IRON METABOLISM IN IRON-DEFICIENT/REPLETE PIGLETS AFTER SUCROSOMIAL® IRON SUPPLEMENTATION

STARZYNSKI Rafal [1], MAZGAJ Rafal [1], KOPEC Zuzanna [1], WANG Xiuying [1], OGLUSZKA Magdalena [1], TARANTINO Germano [3], BRILLI Elisa [3], HERMAN Sylwia [2], KRZYSZTOFIK Daria [2], LENARTOWICZ Malgorzata [2], LIPINSKI Pawel [1]
[1] Institute of Genetics and Animal Biotechnology Polish Academy of Sciences, Magdalenka, Poland; [2] Jagiellonian University, Krakow, Poland; [3] Scientific Department, Pharmanutra S.p.A. , Pisa, Italy

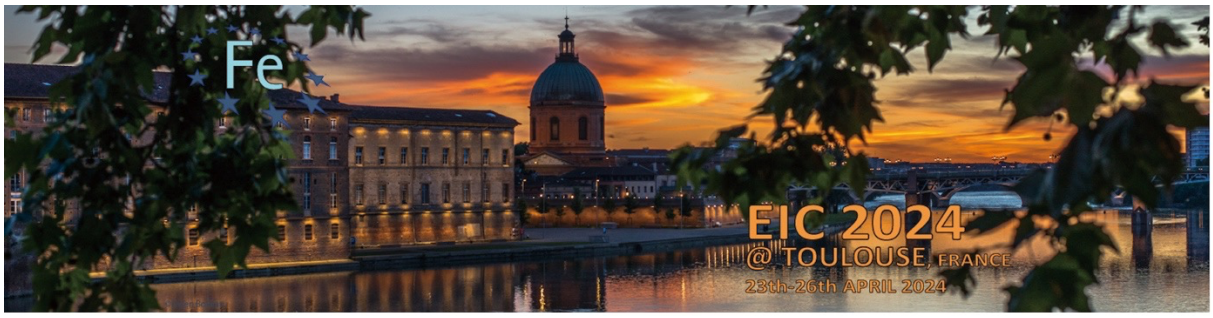
Iron deficiency (ID) is a well-known nutritional disorder, which is widespread in the human population. Oral iron supplementation is a primary approach for the treatment of ID. Sucrosomial® Iron (SI), a highly bioavailable dietary iron suplement is considered a valid option for this therapy. Our aim was to investigate in a time-course study, iron fluctuations in the blood plasma, its tissue content/distribution and iron metabolism genes expression after administration of a single portion of SI to 14-day-old iron-deficient and iron-replete piglets. Our results revealed essential differences in SI-derived iron pharmacokinetics between iron-deficient and iron-replete animals such as faster increase in plasma iron level and its accelerated clearence from the plasma under shortfall compared to iron abundant conditions. Hepcidin plasma level, which predicts iron bioavailability reached a maximum at 6 hours post-administration of SI only in iron-replete piglets. Accordingly, 24 hours after SI administration duodenal ferroportin, the only known iron exporter, remained consistantly at low protein level in this animals whereas in iron-deficient piglets, showed a significant increase. Consequently, at the same time-point, staining for non-heme iron in duodenum showed its massive accumulation in enterocytes of iron-replete piglets suggesting decreased iron absorption in these animals. Our results emphasize the need to closely link the use of SI with iron status of supplemented individuals and predict the involvement of hepcidin in the regulation of SI absorption. Supported by NCN/2020/39/B/NZ5/02469.



P-15 - QUICK RECOVERY OF MOUSE PUPS FROM ANEMIA AFTER MATERNAL IRON DEFCIENCY DURING PREGNANCY

LIPINSKI Pawel [1], OGLUSZKA Magdalena [1], MAZGAJ Rafal [1], PIRGA Natalia [2], PLONKA Wiktoria [2], HERMAN Sylwia [2], LENARTOWICZ Malgorzata [2], WANG Xiuying [1], KOPEC Zuzanna [1], STARZYNSKI Rafal [1]
[1] Institute of Genetics and Animal Biotechnology Polish Academy of Sciences, Magdalenka, Poland; [2] Jagiellonian University, Krakow, Poland

Pregnancy is a physiological condition frequently associated with iron deficiency (ID). The causes of ID are high iron demand and its insufficient supply due to low preconception maternal iron reserves and inadequate dietary intake. Here, using a mouse model of ID in pregnancy, we attempted to determine in a time-course study (11 postnatal days), molecular mechanisms of postnatal recovery of mouse pups after gestational ID. Females fed low iron diet 2 weeks prior to mating, and throughout pregnancy, and their progeny showed clear symptoms of ID anemia. Switching anemic mothers to an iron-replete diet just after delivery resulted in a progressive recovery of their progeny from IDA. On postnatal day 11, RBC indices and iron plasma parameters of pups born to anemic mothers were close to those of pups born to iron-replete females. Higher hepatic ferroportin (Fpn) level in the offspring of anemic mothers strongly suggests that the liver despite decreased iron content still remains a source of iron to meet erythropoietic demand. To check the role of duodenal Fpn in iron delivery to the circulation in iron-deficient pups, we assessed its localization and expression in the duodenum. Fpn was located along the basolateral membranes of absorptive enterocytes in 3-day-old anemic pups and its expression was strongly up-regulated compared to age-mate iron-replete pups showing virtually no Fpn staining. We propose that relatively quick normalization of RBC status in pups born to anemic mothers is due to the efficient mobilization of iron from both exogenous and endogenous sources. Supported by NCN/2020/39/B/NZ5/02469.

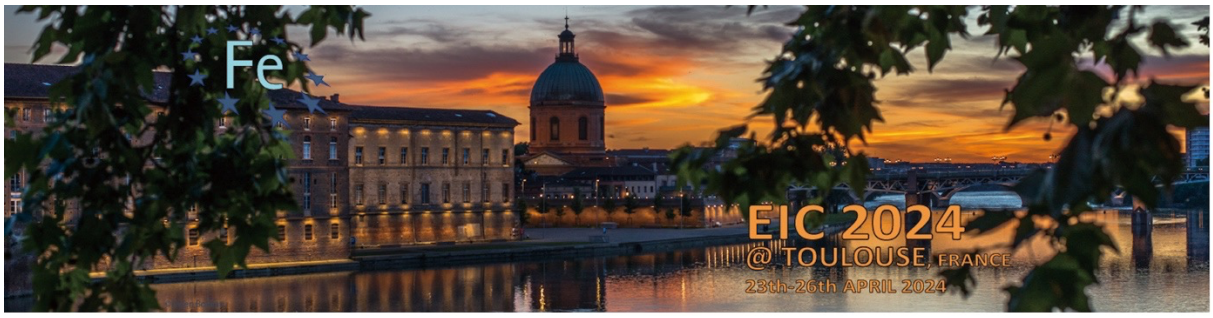


P-16 - ORAL SUCROSOMIAL® IRON IMPROVES IRON STATUS IN PREMATURE PIGLETS BORN BY CESAREAN DELIVERY

WANG Xiuying [1], WOLINSKI Jaroslaw [2], LIPINSKI Pawel [1], OGLUSZKA Magdalena [3], MAZGAJ Rafal [1], KOPEC Zuzanna [1], ZELAZOWSKA Beata [1], TARANTINO Germano [4], BRILLI Elisa [4], **STARZYNSKI Rafal [1]**

[1] Institute of Genetics and Animal Biotechnology PAS, Department of Molecular Biology, Laboratory of Iron Molecular Biology, Jastrzebiec, Poland; [2] Kielanowski Institute of Animal Physiology and Nutrition PAS, Department of Animal Physiology, Laboratory of Large Animal Model, Jablonna, Poland; [3] Institute of Genetics and Animal Biotechnology PAS, Department of Genomics and Biodiversity, Jastrzebiec, Poland; [4] Scientific Department, Pharmanutra S.p.A. Via delle Lenze, Pisa, Italy

Given that the majority of iron transfer from mother to fetus occurs during the third trimester of pregnancy, premature infants are more likely to develop iron imbalances due to inadequate iron storage. Sucrosomial® iron (SI) is an oral iron formulation of ferric pyrophosphate with high bioavailability and tolerability. This research was conducted to evaluate the effects of SI on iron status in premature piglets. Newborn piglets were used in a two-factor factorial design, and the main factors included delivery mode (cesarean section on day 109 of gestation or normal vaginal delivery on day 115 of gestation) and diet (with or without SI). During the experiment, piglets were fed hourly milk. After feeding without or with SI (2 mg Fe/piglet/day) between days 4 and 10 after birth, samples were collected on day 11. Premature piglets showed poor growth performance and low total body iron content. Premature piglets also developed early iron deficiency anemia as indicated by low red blood cell (RBC) count, hemoglobin concentrations and reticulocyte hemoglobin content. These RBC indices were partially improved by SI supplementation. Interestingly, higher hepatic and splenic non-heme iron content were found in preterm piglets compared to full-term piglets. Administration of SI contributed also to the hepatic and splenic iron accumulation in preterm piglets. High tissular iron content in preterm piglets supplemented with SI was associated with increased hepcidin-25 and decreased erythroferrone (its negative regulator) plasma levels. These results indicate that SI attenuates iron deficiency anemia of prematurity. This study was supported by NCN/2020/39/B/NZ5/02469.

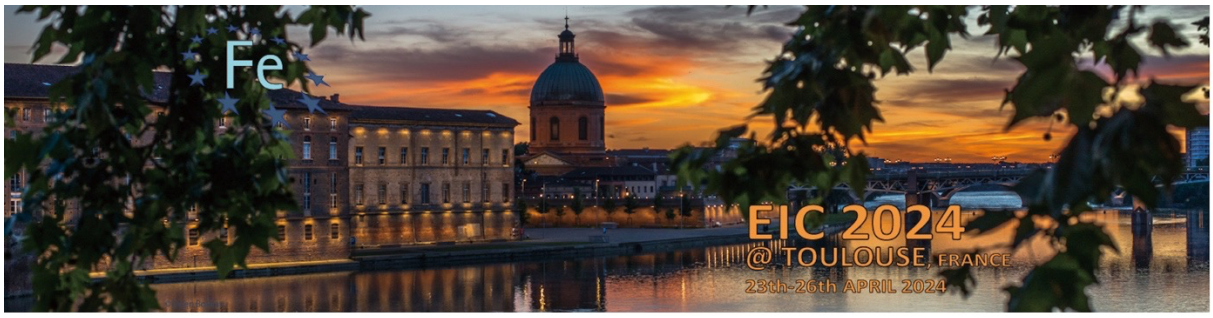


P-17 - PREMATURE BIRTH BY CESAREAN SECTION INFLUENCES IRON METABOLISM IN PIGLETS

WANG Xiuying [1], LIPINSKI Pawel [1], MAZGAJ Rafal [1], OGLUSZKA Magdalena [2], WOLINSKI Jaroslaw [3], SZKOPEK Dominika [3], ZAWORSKI Kamil [3], KOPEC Zuzanna [1], ZELAZOWSKA Beata [1], STARZYNSKI Rafal [1]

[1] Institute of Genetics and Animal Biotechnology PAS, Department of Molecular Biology, Laboratory of Iron Molecular Biology, Jastrzebiec, Poland; [2] Institute of Genetics and Animal Biotechnology PAS, Department of Genomics and Biodiversity, Jastrzebiec, Poland; [3] Kielanowski Institute of Animal Physiology and Nutrition PAS, Department of Animal Physiology, Laboratory of Large Animal Model, Jablonna, Poland

In contrast to adults, our understanding of iron metabolism regulation in early postnatal periods, is poor. This study evaluated and compared iron status in term and preterm newborns using a porcine model. Cesarean section was manipulated on day 109 of gestation to obtain premature piglets. Piglets born naturally on day 115 were used as control animals. Blood, liver and spleen samples were collected during the first 4 hours after full-term and preterm birth. Our previous data showed lower red blood cell indices, plasma iron level and total body iron content in premature piglets compared to term animals, which indicated the occurrence of iron deficiency in prematurity. Here, we provide data showing hepatic iron accumulation, increased mRNA and protein expression of both ferritin chains, and upregulation of cytosolic iron chaperone poly(rC)-binding protein 1 (PCBP1) mRNA expression in the liver and spleen, of preterm piglets. Increased hepatic iron status of preterm piglets was associated with a statistically significant upregulation of hepcidin mRNA level. In parallel, in prematurely born piglets we observed orchestrated changes in the expression of hepcidin regulators such as increase in hepatic bone morphogenetic protein 6, and decrease in erythropoietic factors—erythroferrone and growth differentiation factor 15 in prematurely. It seems that prematurely born piglets obtained by cesarean section show pattern of iron metabolism characteristic for functional iron deficiency and iron accumulation in the tissue. It is possible that up-regulation of hepatic hepcidin is one of factors causing iron imbalance in preterm neonates. This study was supported by NCN/2020/39/B/NZ5/02469.



P-18 - EXPLORING THE CROSSTALK BETWEEN IRON HOMEOSTASIS AND ADIPOSE METABOLISM

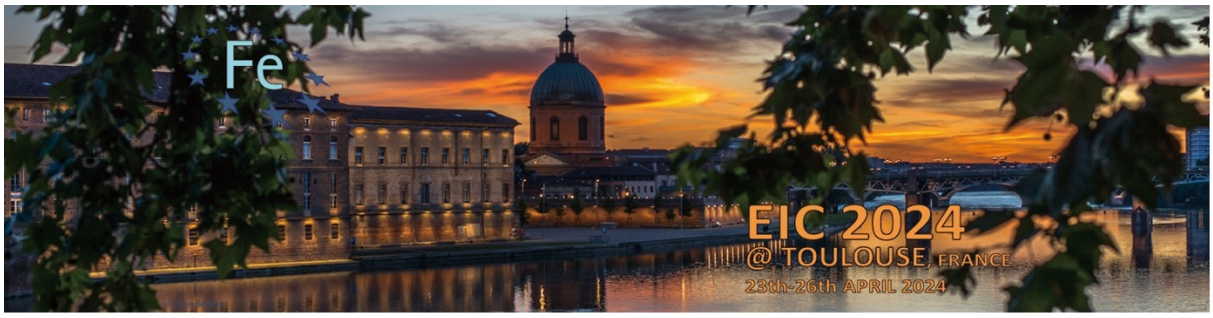
KARIN Gabriel [1], SANGKHAE Veena [1], NEMETH Elizabeta [1], GANZ Tomas [1]
[1] UCLA Center for Iron Disorders, Los Angeles, United States

The normal functioning of many tissues relies on the tight regulation of both iron concentration and distribution within cells and tissues. Studies have shown that changes in adipocyte iron alter several aspects of adipose metabolism. Here we focus on understanding how Tmprss6, a hepatic serine protease, influences lipid metabolism and its potential implications for treating obesity and metabolic disorders. The loss of Tmprss6 in mice is protective against diet-induced obesity and promotes lipolysis; however, the mechanism is unclear. Utilizing a GalNac-conjugated Tmprss6 antisense oligonucleotide (ASO) to achieve Tmprss6 deficiency in mice, revealed a stark reduction in fat mass. Two hypotheses are being explored to understand the mechanisms behind the fat-reducing effects of Tmprss6-ASO:

Hepcidin-Dependent Mechanism:
- Tmprss6-ASO induces hepcidin, retaining iron in adipose stromal vascular fraction (SVF) macrophages. This reduces iron availability to adipocytes lowering lipid absorption in the gut.
- The study aims to confirm the necessity of hepcidin for the observed fat-reducing effects using hepcidin knockout (KO) mice.

Hepcidin-Independent Mechanism:
- As a type II transmembrane serine protease (TTSP), Tmprss6 may activate hepatocyte growth factor (HGF). Activated HGF serves as a hepatokine linked to adipose metabolism.
- Here we investigate whether Tmprss6-ASO inhibits the proteolytic activation of HGF, leading to reduced body fat by reducing adipogenesis in various adipose depots.

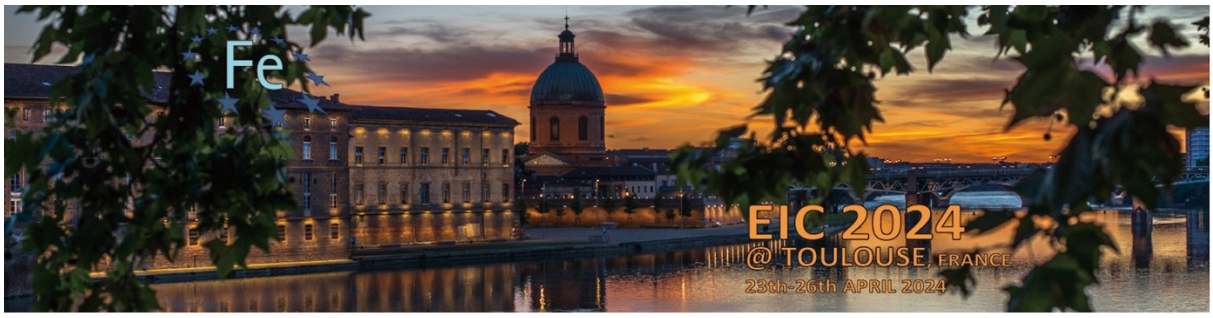
In summary, the study seeks to determine whether Tmprss6 influences lipid metabolism through hepcidin or independently via altered adipocyte HGF/c-Met signaling. Understanding these mechanisms could unveil novel therapeutic targets for obesity and metabolic disorders.



P-19 - MECHANISTIC INSIGHTS ON DIETARY IRON ABSORPTION: THE ROLES OF HEPCIDIN AND TRANSFERRIN

KATSAROU Angeliki [1], TSYPLENKOVA Sofiya [1], CHARLEBOIS Edouard [1], CARINE Fillebeen [1], **PANTOPOULOS Kostas [1]**
[1] Lady Davis Institute for Medical Research and McGill University, Montreal, Canada

Hepcidin is a liver-derived peptide hormone that controls systemic iron homeostasis by inhibiting iron entry into plasma. Plasma iron is captured by transferrin for delivery to the bone marrow and other tissues. We studied the effects of hepcidin or transferrin injection on dietary iron absorption. First, wild type and Hjv-/- mice, a model of hemochromatosis, were injected with synthetic hepcidin. A dose of 2 g/kg synthetic hepcidin reached maximal plasma concentration of 1.3 µg/ml, with 90 min half-life. Hepcidin partially decreased levels of ferroportin and the apical metal transporter DMT1 in wild type duodenal enterocytes. Surprisingly, hepcidin profoundly suppressed duodenal DMT1 in Hjv-/- mice without affecting highly induced ferroportin. Nevertheless, the treatment triggered duodenal iron retention. Experiments in intestinal mouse organoids showed that ferroportin and DMT1 are sensitive to degradation by hepcidin or iron, respectively. Thus, hepcidin efficiently degrades basal duodenal ferroportin but appears limiting when ferroportin is overexpressed. Nevertheless, under these conditions, hepcidin occludes ferroportin's iron-exporting channel and causes iron accumulation in enterocytes. Our data show that iron retention due to hepcidin-mediated ferroportin inactivation is the signal that drives DMT1 degradation in enterocytes. In a second set of experiments, iron-deficient wild type mice were injected with apo-transferrin and were switched to a high-iron diet. Unexpectedly, excess apo-transferrin enhanced dietary iron absorption and triggered accumulation of plasma non-transferrin bound iron (NTBI). Injected fluorescent-labeled transferrin colocalized with lamina propria macrophages. These data are consistent with a recently proposed iron absorption checkpoint involving macrophage-mediated transferrin degradation.



P-20 - CHARACTERIZATION OF THE METALLOME OF EXTREME PHYSICAL INACTIVITY IN HUMANS AND RODENTS

HOREAU Mathieu [1], NAVASIOLAVA Nastassia [3,4], VAN OMBERGEN Angelique [5], CUSTAUD Marc-Antoine [3,4], ROPERT Martine [2], ANTUNES Ines [6], BAREILLE Marie-Pierre [7], GAUQUELIN-KOCH Guillemette [8], BILLETTE DE VILLEMEUR Rebecca [7], LORÉAL Olivier [2], DERBRÉ Frédéric [1]

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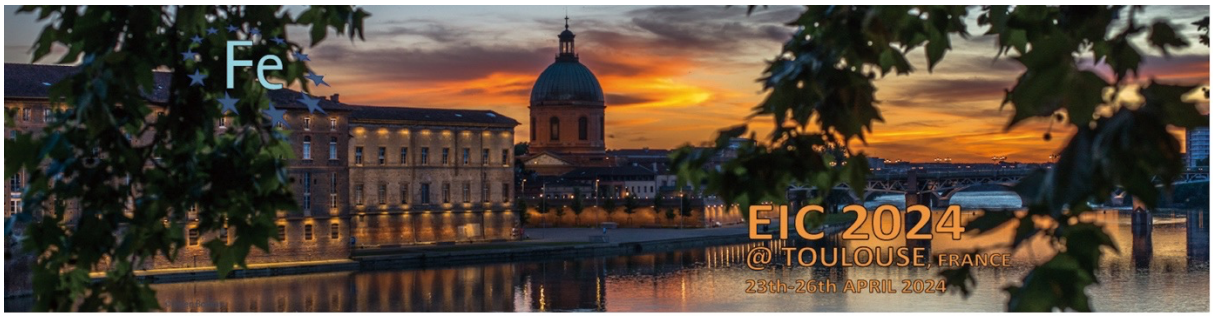
This study aims to characterize, in humans and rodents, the response of the metallome to experimental models of extreme physical inactivity (EPI), specific to astronaut and bedridden patients, with a special focus on sex-related differences. Using Inductively Coupled Plasma/Mass Spectrometry, we analyzed six essential metals that constitute the plasma metallome of men and women exposed to 5 days of dry immersion (DI) and male and female rats subjected to 7 days of hindlimb unloading (HU). In rats, we also investigated the metallomic fingerprints of the liver, spleen, and skeletal muscles to identify potential metal redistributions.

At baseline, women had higher plasma Cu levels than men. In rats, females exhibited higher plasma levels of Fe and Cu, and lower concentrations of Mn. Under DI, plasma Fe and Zn levels increased in both sexes, and Cu levels increased only in men. In rats, HU resulted in elevated plasma Cu and Se levels in both sexes. Principal Component Analysis revealed organ and muscle type-specific metallomes in control groups. In the soleus, concentrations of Fe, Mg, Se, and Mn increased in HU females. In males, HU did not affect soleus Fe concentrations and promoted a decrease in Mg and Mn levels. In the gastrocnemius, irrespective of sex, HU led to increased Mg, Se, and Mn concentrations, with Cu, Zn, and Fe concentrations not significantly affected. In both sexes, the liver and spleen metallome was substantially affected by EPI.

Our data suggest that EPI profoundly affects plasma and organ metallome in both humans and rodents.



P-21 - Extreme physical inactivity affects differentially the iron metabolism in male and female rats

HOREAU Mathieu [1], MARTIN Brice [1], ORFILA Luz [1], ROPERT Martine [2], LEROYER Patricia [2], LORÉAL Olivier [2], DERBRÉ Frédéric [1]

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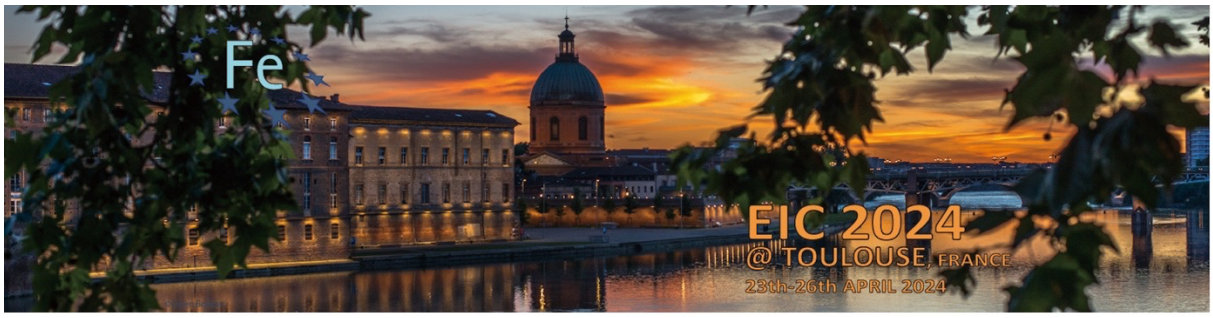
In humans, extreme physical inactivity (EPI) appears to differentially affect iron metabolism between sexes. Our objective was to understand the mechanisms involved in rats of both sexes exposed to hindlimb unloading (HU), an experimental model mimicking EPI. Eight-week-old male and female Wistar rats (n=12/group) underwent 7 days of HU, with control groups included. Serum, liver, spleen, and soleus muscle samples were collected. ICP-MS analyses were used to quantify iron concentration. Gene expression was analyzed by RT-qPCR and Western Blot.

In contrast to humans, control females exhibited higher serum iron availability, hepatic and spleen iron concentrations (HIC and SIC) than males. In HU males, serum iron availability was not affected, whereas there was an increase in serum hepcidin level, SIC, and HIC ($p=0.001$; $p=0.023$; $p=0.003$, respectively). Moreover, spleen and liver ferritin protein levels (FPL) increased (+60.9% and +134%, respectively; $p<0.05$), while TfR1 protein levels decreased (-50%; -35%, respectively; $p<0.05$). In HU females, there was no significant change in serum hepcidin level, LIC, SIC, and regarding TfR1 protein and FPL in spleen and liver. In HU males, concomitantly with SIC increase, heme oxygenase-1 mRNA level, a marker of RBC phagocytosis, increased ($p<0.001$). Paradoxically, it also increased in HU females ($p<0.001$). Alongside muscle atrophy observed in HU rodents, iron concentrations and FPL increased in the soleus in both sexes ($p<0.001$, $p=0.047$), and TfR1 protein was reduced ($p<0.001$), while muscle myoglobin protein and heme exporter FLVCR1 mRNA levels increased ($p<0.001$).

Our data suggest that EPI induces iron misdistribution only in males through accelerated erythrophagocytosis



P-22 - QUALITY OF LIFE AND FUNCTIONAL RESPONSE TO PERI-OPERATIVE ADMINISTRATION OF INTRAVENOUS IRON FOR THE

MACLEAN Beth [1,2], LIM Jayne [1], CLEMENT Ines [2], HINES Elizabeth [2], RICHARDS Toby [1,2,3]
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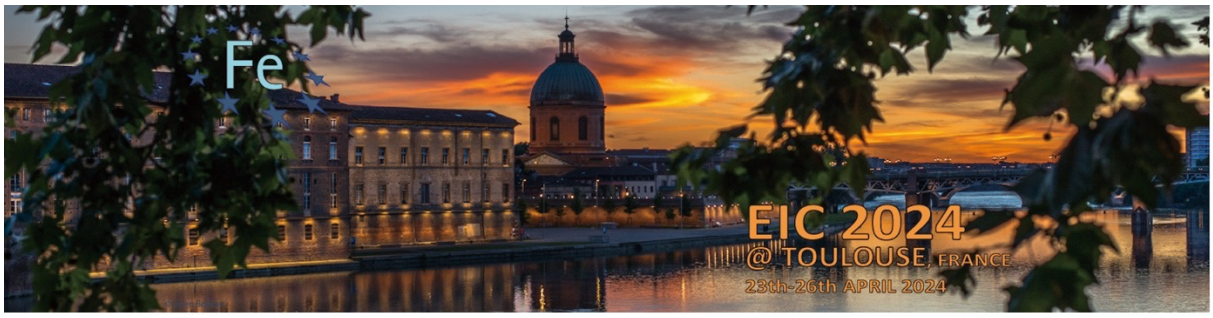
Introduction
In major surgery, it is unclear whether the use of intravenous (IV) iron to treat anaemia can provide a direct benefit to patients. We aimed to assess the effect of IV iron administration during major surgery on muscle function and quality of life (QoL) outcomes.

Methods
Adult patients with anaemia, defined as haemoglobin concentration (Hb) <120g/L for women and <130g/L for men, undergoing major lower limb vascular surgery were included from a single centre. Patients were randomised 1:1 in a double-blinded manner, to receive 1000mg of ferric carboxymaltose (FCM) or placebo (saline). The primary outcome was Hb at 30-days post-operation. Secondary outcomes included QoL assessments (EQ-5D-5DL and SF-36) and hand grip strength (GS). Trial registration: ACTRN12622001447741.

Results
Of 26 recruited patients:12 were allocated to FCM and 14 to placebo. Mean age was 64.7 ±11.7years and 15% were female. Baseline Hb was similar (FCM: 107.6 ±15.8g/L vs placebo: 113.9 ±16.0g/L, p=0.327), with no difference by 30-days post-operation (109 ±9.6g/L vs 112.1 ±17.7g/L, p=0.609). By day 30, there was no difference in GS (26.6 ±13.6 vs 25.8 ±14.3, p=0.918). From day 8 to 30, no significant difference was observed between groups in change of GS (-6.4 ±18.5 vs -14.1 ±15.9, p=0.270) nor EQ-5D-5L visual analogue score (-12.4 ±33.8 vs 3.14 ±24.1, p=0.720). No difference was observed in any QoL components between the groups.

Conclusions
The use of IV iron peri-operatively did not improve Hb, QoL outcomes or GS by 30 days post-operation. Long-term re-evaluation of these outcomes is required.



P-23 - DEVELOPING A SCREENING TOOL FOR IRON DEFICIENCY ANAEMIA DETECTION IN WOMEN DURING REPRODUCTIVE years

MACLEAN Beth [1], LIM Jayne [1], FULLER Jess [1], WYLIE Riki [1], JOO Judie [1], AL-SHAREA Annas [1], CHEYYUR Jaahnavi [1], NG Henry [1], DUGAN Cory [2], RICHARDS Toby [3]

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Introduction

Women are at an increased risk of developing an iron deficiency during their reproductive years. Iron deficiency can result in a plethora of non-specific symptoms, providing difficulties for early detection. We aimed to develop a non-invasive screening tool to identify women at risk of an iron deficiency.

Methods

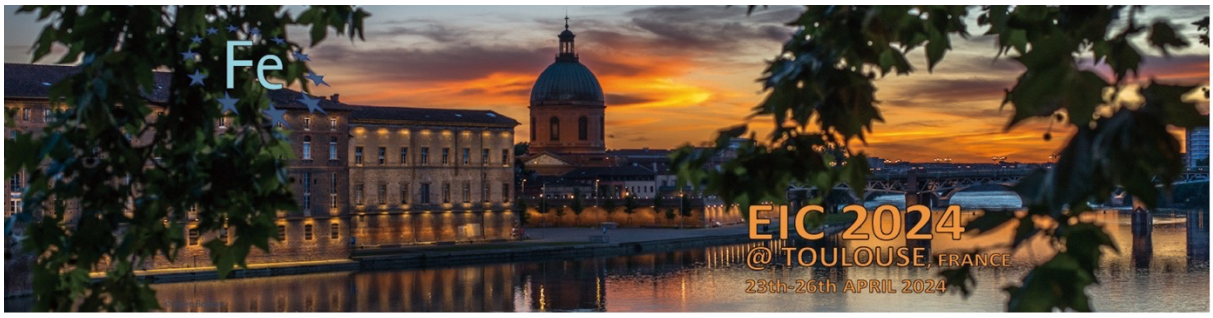
Women aged 18 – 49 years were recruited from a shopping centre in Western Australia. Questionnaires captured relevant history on menstruation, diet, pregnancy, blood donation and any prior diagnosis or treatment for iron deficiency or anaemia. Finger prick testing was conducted to assess haemoglobin concentration (Hb) and a hand grip dynamometer measured grip strength.

Results

Of the 387 women, 45 (12%) were anaemic (Hb <120g/L) and 110 (28%) had symptoms of heavy menstrual bleeding (HMB). Mean age was 33.2 ±9.2years and mean Hb was 132.1 ±12.1g/L. In the past two years: 49% had a diagnosis of iron deficiency or anaemia; 49% had taken oral iron; and 21% had an intravenous iron infusion. Vegetarian diets were followed by 13%; 6% were regular blood donors; 47% had a previous pregnancy; these factors did not influence Hb. Those with anaemia reported more periods in the past 12 months (11 ±2.4 vs 9.8 ±3.8, p=0.005). Linear regression analysis showed handgrip strength was associated with Hb (p=0.044, R2=0.008), furthermore, there was a greater prevalence of sarcopenia (grip strength <16kg) in the anaemic subgroup (4% vs 1%, p=0.038).

Conclusions

Hand grip strength and period frequency could be valuable for incorporation into an iron deficiency anaemia screening tool.

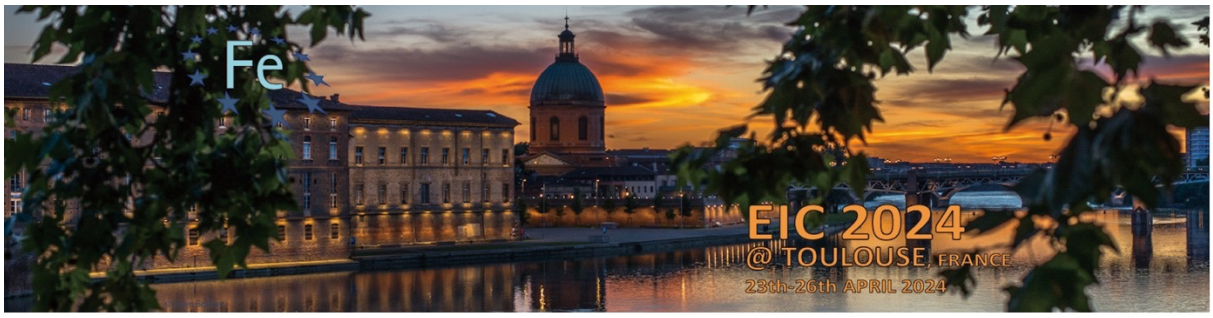


P-24 - SLC7A7 DEFICIENCY CAUSES RENAL DYSFUNCTION AND REDUCED ERYTHROPOIETIN COMPROMISING ERYTHROPOIESIS

GIROUD-GERBETANT Judith [2], SÁNCHEZ FERNÁNDEZ Mayka [3], WEISS Gunter [4], PALACÍN Manuel [2,5], **BODOY Susanna** [1,2,5]

[1] Universitat de Vic – Universitat Central de Catalunya, Vic, Spain; [2] IRB Barcelona, Barcelona, Spain; [3] Universitat Internacional de Catalunya, Sant Cugat, Spain; [4] Medical University of Innsbruck, Innsbruck, Austria; [5] Centro de Investigación Biomédica en Red en Enfermedades Raras , Madrid, Spain

Lysinuric Protein Intolerance (LPI) is an inborn metabolic disorder caused by mutations in SLC7A7, a cationic amino acid transporter leading to reduced plasma levels of cationic amino acids and urea cycle abnormalities. Clinical manifestations in patients are diverse, often involving severe hematological, immunological anomalies, and kidney failure. Our investigation demonstrates that Slc7a7 deficiency in mice causes iron overload, erythropoiesis dysfunction, and diminished erythrocyte size. We ruled out a direct intrinsic impact of Slc7a7 on erythroid or myeloid lineages using cell-lineage-specific mouse models, focusing on the systemic environment as the main player in the mice's phenotype. Regarding iron metabolism, we suggest that reduced plasma erythropoietin triggers a significant iron overload, as erythropoietin administration restores normal iron levels and mitigates hematological alterations. Notably, we observed kidney failure in LPI mice, mirroring the clinical scenario in LPI patients. Recovery experiments with citrulline treatment were able to restore kidney cortical mitochondrial respiration, prevent renal fibrosis, and recover EPO levels. Interestingly, human LPI is associated with hyperferritinemia but not iron overload, a trait that might be obscured by citrulline treatment. This study unveils a previously unrecognized role of EPO in the disease and suggests EPO as a promising therapeutic strategy for LPI patients.

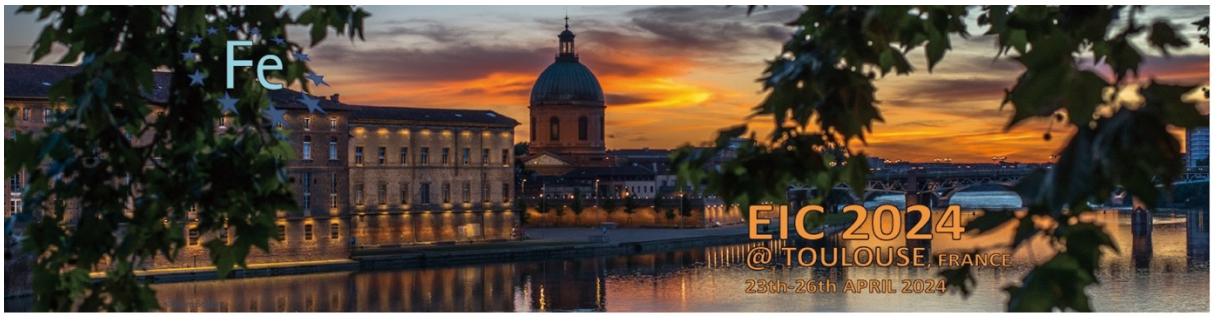


P-25 - LENTIVIRAL GENE THERAPY RESCUES A NOVEL INDUCIBLE CODANIN-1 KO MOUSE MODEL OF BONE MARROW FAILURE

DEMSKO Perry [1,2], GUERRA Amaliris [1], BREDA Laura [1], CASTRUCCIO CASTRACANI Carlo [1], RIVERA Ariel [1], PAPP Tyler [2], TAM Yink [3], MUI Barbara [3], FEDORKY Megan [1], PARHIZ Hamideh [2], **RIVELLA Stefano [1,2]**

[1] Children's Hospital of Philadelphia, Philadelphia, United States; [2] University of Pennsylvania, Philadelphia, United States; [3] Acuitas Therapeutics, Vancouver, Canada

Congenital Dyserythropoietic Anemia 1 (CDA1) is an inherited blood disorder caused by biallelic mutations in Codanin-1 (Cdan1). Complications from CDA1 are primarily derived from deficient erythropoiesis and include splenomegaly, hemochromatosis, and hyperbilirubinemia, which are further exacerbated by chronic transfusion dependence. Currently, no cure is available, and the lack of a viable CDA1 mouse model has prevented attempts to develop treatments. We have developed two CDA1 models wherein a floxed Cdan1 allele is excised either by Mx-Cre after induction with a dsRNA analog (Cdan1Mx-cre-KO) or via Cre mRNA containing lipid nanoparticles targeted to hematopoietic stem cells (Cdan1LNP-KO). The fatal phenotype can be reversed by rescuing the model by delivering the human CDAN1 gene via lentiviral gene transfer (AP-CDAN1-FWP). All surviving KO animals show normal erythropoiesis and complete blood counts. Additional studies are underway to analyze lineage-specific hematopoiesis in the bone marrow. Since hematopoietic stem cells lacking Cdan1 are not viable, we generated mice KO with transgenic Cdan1 using vectors expressing known hypomorphic human CDAN1 mutations. In early studies, and similarly to human CDA1 patients, the mice transduced with mutated CDAN1 alleles exhibit variable decreases in hemoglobin and red blood cell count. In particular, the anemic state is more prominent up to 4 weeks following transplant or a challenge bleed, with CDAN1 mutant mice exhibiting a protracted recovery compared to WT controls. Experiments are underway to characterize erythropoiesis and iron metabolism in these animals. These data indicate that our novel vectors can potentially cure CDA1 and further characterize this disease.



P-26 - EXPANDING THE MOLECULAR MECHANISMS AND THE PATIENT COHORT IN CONGENITAL DYSERYTHROPOIETIC ANEMIA III

HERNANDEZ Gonzalo [1,2], TOMAS Pau [2], SAVARINO Mario-Enrique [3], QUIJANO Sandra [3], PUJAL Graciela [3], TORNADOR Cristian [2], **SANCHEZ Mayka [1,2]**

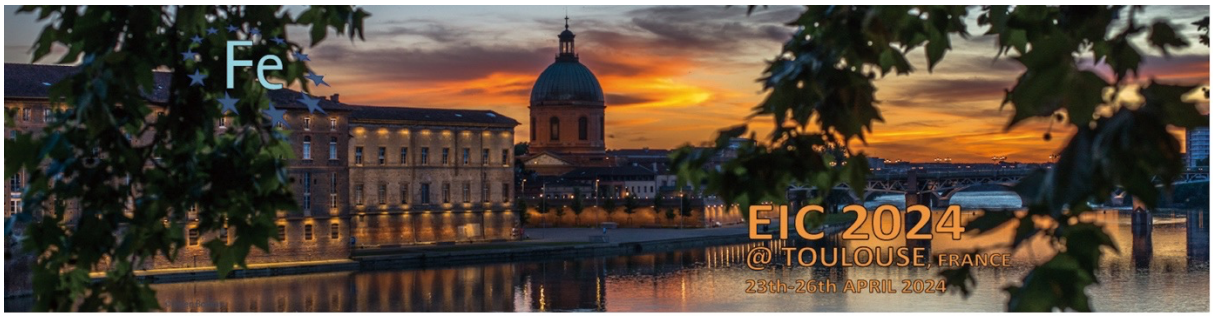
[1] Department of Basic Sciences, Iron metabolism: Regulation and Diseases Group. Universitat Internacional de Catalunya (UIC), Barcelona, Spain; [2] BloodGenetics S.L. Diagnostics in Inherited Blood Diseases. , Esplugues de Llobregat, Spain; [3] Hospital J.C.Perrando. , Resistencia, Chaco, Argentina

Background: Congenital Dyserythropoietic Anemia type III (CDA III), the rarest subtype in the CDA spectrum, includes autosomal dominant CDA IIIa with monoallelic KIF23 mutations and recessive CDA IIIb with biallelic RACGAP1 missense mutations, as we recently shown. Limited documented cases hinder a complete understanding of the syndrome, posing challenges in accurate diagnosis due to its rarity.

Aims: To report new patients affected by CDAIIIb and characterize the cellular and clinical abnormalities associated with mutations in the RACGAP1 protein.

Methods: DNA samples were collected from five Argentinian individuals with clinical features suggestive of CDAIIIb, along with healthy relatives. We conducted targeted Next-Generation Sequencing (NGS) using a comprehensive panel covering all CDA-associated genes. In addition, in vitro assays with cell lines were performed to assess the impact of missense mutations in the RACGAP1 protein. These assays focused on evaluating the protein's centralspindlin complex formation ability and its capacity to modulate key GTPases essential for cytokinesis progression.

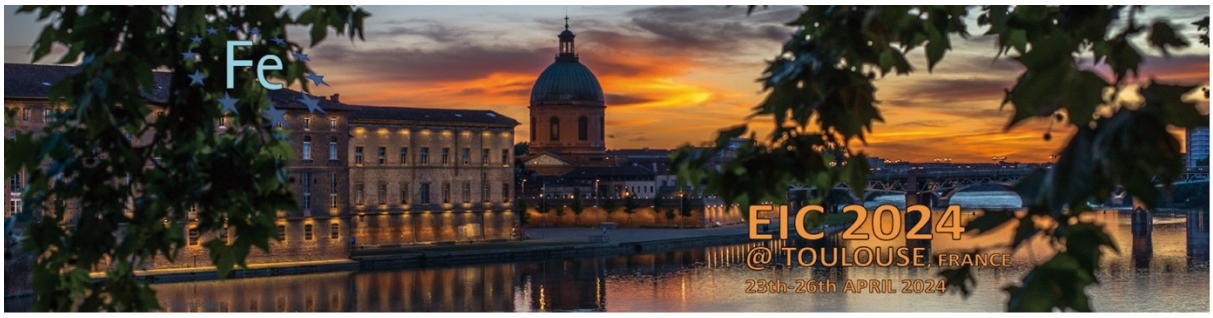
Results: All patients displayed typical CDAIII symptoms, including macrocytic anemia, hemolysis (increased bilirubin and LDH with absence of haptoglobin), and giant multinucleated erythroblasts in the bone marrow. Surprisingly, all affected individuals were homozygous for the known Pro432Ser mutation, while healthy relatives were carriers. In vitro findings reveal that the Pro432Ser and Thr220Ala mutations do not hinder centralspindlin complex formation or localization. However, they disrupt its ability to accurately modulate the activation status of RHOA, CDC42, and RAC1 GTPases.
Supported by grants PID2021-122436OB-I00 and grant RTC2019-007074-1 from MCIN/AEI /10.13039/501100011033 to MS.



P-27 - THE ROLE OF IRON IN THE INFLAMMATORY STAGE OF HETEROTOPIC OSSIFICATION

SPANGENBERG Sven [1], HOFBAUER Lorenz [1], RAUNER Martina [1], BASCHANT Ulrike [1]
[1] Department of Medicine III & Center for Healthy Aging, Medical Faculty and University Hospital Carl Gustav Carus, Dresden University of Technology, Dresden, Germany

Heterotopic ossification (HO) is bone formation at abnormal anatomical sites, developing in three main stages: inflammation, chondrogenesis and osteogenesis. As iron homeostasis plays an important role in inflammatory diseases and bone formation, we aimed to investigate the impact of iron levels on HO development. For this purpose, wild type mice were fed with either low (<10 ppm Fe), normal (200 ppm Fe) or high (25,000 ppm Fe) iron diet for 6 weeks starting from weaning on. Afterwards, HO was induced by injection of recombinant BMP-2 into the M. tibialis anterior. HO sites were analyzed by flow cytometry, proteome cytokine profiler (day 3) and µCT analysis (day 14). In mice fed with high iron diet, iron accumulated in the inner organs such as liver (2001.6 vs 280.7 µg Fe/g dry tissue, $p<0.001$) as well as in the muscle (0.14 vs 0.69 µg Fe/ mg protein, $p<0.001$). High iron diet led to a 2.6-fold increase in HO 14 days after BMP-2 injection ($p<0.05$), while low iron diet led to a 65 %-decrease of bone formation ($p<0.05$) compared to normal iron diet. Analyzing the inflammatory phase of HO, mice under high iron diet showed enhanced expression of pro-inflammatory cytokines (e.g. IFNγ: 2-fold increase; $p<0.05$, IL-6: 1.7-fold; $p<0.05$) and more infiltrated CD11b+ myeloid cells and classical macrophages ($p<0.05$). Neutrophils and non-classical macrophages were decreased under high-iron diet ($p<0.05$). Iron levels affect the development of HO, most likely already in the early inflammatory stage of HO by increasing pro-inflammatory cytokines and immune cells.



P-29 - PLACENTAL PROTEINS INVOLVED IN IRON HOMEOSTASIS WERE DOWNREGULATED IN GESTATIONAL DIABETES MELLITUS

JOHN Nikhitha Mariya [1], LANGBEIN Jannik Leon [1], BECK Manisha [1], RATHORE Swati [1], **JACOB Molly** [1]
[1] Christian Medical College, Vellore, India, Vellore, India

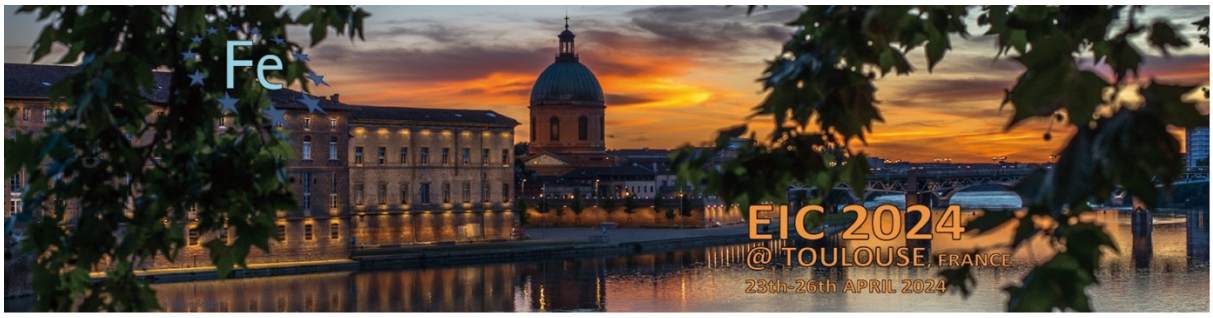
Background: High iron status in pregnancy has been associated with increased risk of gestational diabetes mellitus (GDM). Hyperglycaemia in GDM has been suggested to affect processes involved in placental iron homeostasis, but it is unclear whether these affect transfer of iron to the fetus.

Objectives: To compare maternal and fetal iron status, and placental proteins involved in iron homeostasis, in women with and without GDM

Methods: Primigravidae, with and without GDM, were followed up through pregnancy until delivery. Hematological and iron-related parameters were estimated in maternal and cord blood samples. Placental tissue obtained was used to estimate tissue iron content and to determine gene expression of proteins involved in iron homeostasis.

Results: Women with GDM (n = 30) and those without (n = 77) had similar sociodemographic and clinical characteristics; hematological and iron-related parameters in maternal blood were similar in the 2 groups. In cord blood from mothers with GDM, reticulocyte count and serum ferritin values were lower than in those without (p values = 0.027 and 0.055 respectively). Gene expression of placental ferroportin, ferritin-H and ZIP8 were significantly lower in those with GDM than in those without. Other relevant genes and iron content in the placentae were similar in both groups.

Conclusions: Placental expression levels of proteins involved in iron homeostasis were lower in women with GDM than in those without, implying less transfer of iron to the fetus in these women. Findings of lower levels of serum ferritin and reticulocyte counts in the cord blood corroborate this observation.

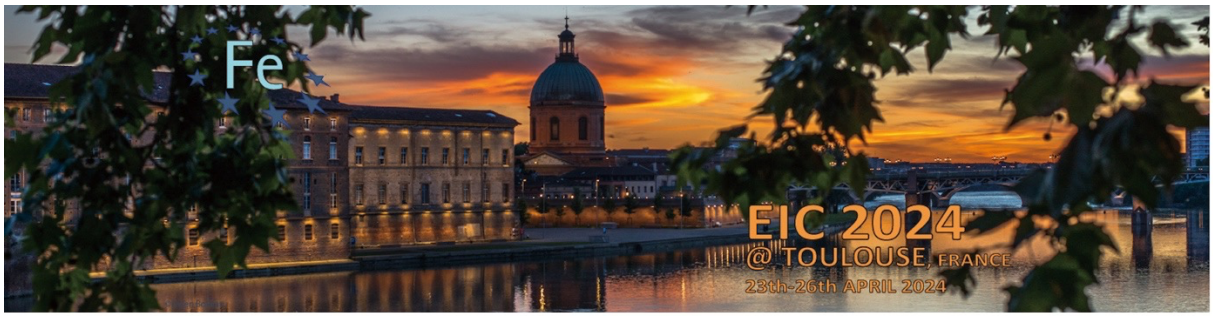


P-30 - Iron absorption and iron gains from birth to 6 months in breastfed and formula fed infants

STOFFEL Nicole [1,2], CEPEDA-LÓPEZ Ana Carla [3], ZEDER Christophe [2], HERTER-AEBERLI Isabelle [2], ZIMMERMANN Michael [1]

[1] University of Oxford, Oxford, United Kingdom; [2] ETH Zurich, Zurich, Switzerland; [3] Tecnologico de Monterrey, Monterrey, Mexico

Many infants develop iron deficiency by 6 months of age. Yet little is known about iron absorption or iron gains in early infancy. We used a novel isotope dilution method to measure dietary iron absorption from birth to 6 months in healthy term infants who were fully breastfed (BF), fully formula-fed (FF) or received mixed feeding (MF). In this prospective observational study, we administered stable iron isotopes (57Fe, 58Fe) to pregnant women and used maternal-fetal iron transfer to uniformly label the newborn. Dilution of enriched infant body iron by dietary iron with natural isotopic composition was used to quantify iron absorption from birth to 6 months and calculate iron bioavailability and iron gains. In BF infants (n=8), FF infants (all receiving high-iron formula) (n=7) and MF infants (n=8), median (IQR) iron absorbed was 0.128 (0.095–0.180), 0.457 (0.374–0.617) and 0.391 (0.283–0.473) mg/day (BF vs FF, $p<0.01$); dietary iron bioavailability was 42.3 (20.4–52.7), 3.2 (2.5–7.4) and 7.3 (6.4–11.3)% (BF vs FF, $p=0.001$; BF vs MF, $p<0.05$); and total iron gains were 0.027 (-0.002–0.055), 0.349 (0.260–0.498) and 0.276 (0.175–0.368) mg/day (BF vs FF, $p<0.001$; BF vs MF, $p<0.05$). In BF infants, iron absorbed just covered basal iron losses, but resulted in minimal iron gains; as a result, hemoglobin, total body iron and circulating iron were lower than in FF infants at 6 months (for all, $p<0.01$). Most infants (20 of 23) absorbed much less iron then the recommended 0.7 mg/day for 6-month-olds.



P-31 - Estrogens and Prolactin Do Not Regulate Maternal and Embryo Iron Homeostasis During Pregnancy

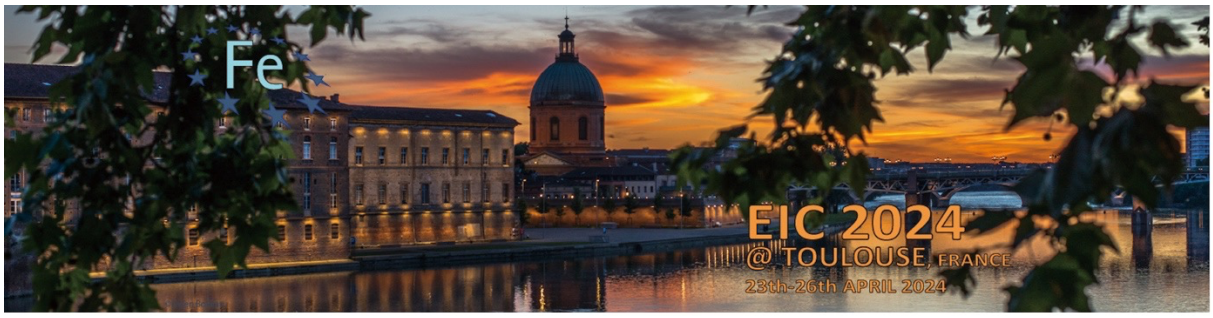
ZHANG Vida [1]

[1] University of California, Los Angeles, ,

Iron is essential for maternal and fetal health but the mechanisms ensuring increased iron availability during pregnancy are unclear. Hepcidin is the key iron-regulatory hormone and reduces circulating iron levels by preventing iron absorption and mobilization. In healthy human and rodent pregnancies, maternal hepcidin decreases starting in the second trimester to nearly undetectable levels by late pregnancy, which allows for greater iron availability to the fetus. Inappropriately high maternal hepcidin levels during pregnancy results in fetal anemia, intrauterine growth restriction, and potentially fetal death.

Estrogens and prolactin have previously been implicated in baseline hepcidin regulation. As the levels of both hormones increase dramatically over the course of pregnancy, we hypothesized that they may modulate iron homeostasis during pregnancy. Estrogen and prolactin are required for pregnancy and global knockout (KO) of either estrogen receptor alpha (ER-α) or prolactin receptor (PRLR) results in infertility. Thus, to determine the contribution of estrogens and prolactin on maternal hepcidin suppression during pregnancy we generated mice with liver-specific knockouts of either ER-α (Er-αf/f;Alb-Cre+, ER-α KO) or PRLR (Prlrf/f;Alb-Cre+, PRLR KO).

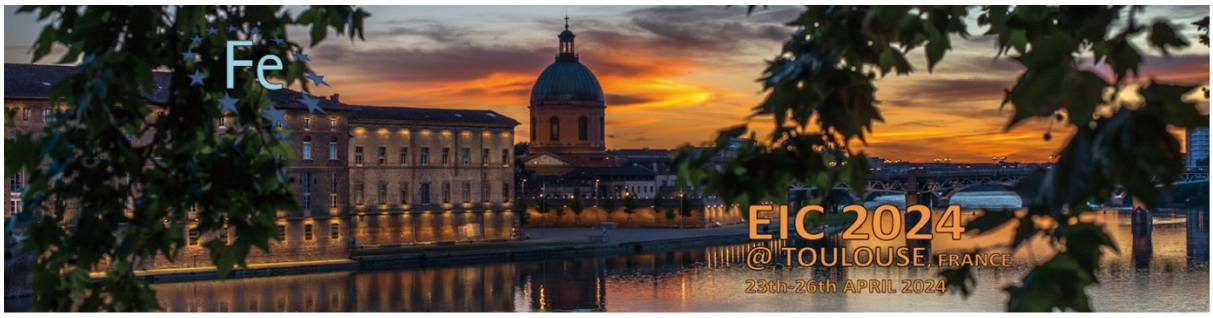
ER-α KO, PRLR KO and wild-type (WT) females were set up for timed pregnancy and analyzed at E18.5. We observed no change in liver hepcidin mRNA levels in ER-α KO and PRLR KO dams compared to WT dams. ER-α KO, PRLR KO and WT pregnancies also displayed comparable maternal and embryo hematological and iron parameters. These data indicate that estrogens and prolactin do not play a role in maternal hepcidin suppression or iron regulation during pregnancy.



P-32 - ASSOCIATION OF FERRITIN AND PLASMA IRON LEVELS AT TIME OF VACCINATION WITH THE IMMUNE RESPONSE TO SA

PESTONI Giulia [1], MENGES Dominik [2], SCHEUCHZER Pornpimol [1], BRAUN Julia [2], HAILE Sarah [2], BALLUZ Tala [2], ZEDER Christophe [3], STOFFEL Nicole [2,3], ZIMMERMANN Michael [2,3], FREI Anja [2], PUHAN Milo [2], **MORETTI Diego [1]**
[1] University of Applied Sciences of South Switzerland (SUPSI) / Swiss Distance University of Applied Sciences (FFHS), Zürich, Switzerland; [2] Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zürich, Switzerland; [3] Laboratory of Nutrition and Metabolic Epigenetics, Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland; [4] Medical Research Council Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

Background: Iron status may affect the immune response following vaccination. We aimed to investigate whether ferritin and plasma iron at time of vaccination were associated with the development and temporal decay of immune response to SARS-CoV-2 vaccination over 26 weeks in the population-based Zurich SARS-CoV-2 Vaccine cohort. Methods: Participants (n=572) were randomly recruited stratifying by age groups (18-64years, >65 years) and vaccine types (Pfizer-BioNTech BNT162b2, Modernam RNA-1273, Johnson&Johnson JNJ-78436735). Blood samples and self-administered questionnaires were collected at baseline and 4, 6, 13, and 26 weeks. Iron status was measured at baseline, whereas immunity markers at each time point. The association between iron parameters and immunity markers was investigated using linear mixed-effect models. Half-life was estimated using log(0.5)/model-coefficient. Results: Mean age(±SD) was 56.0±18.1 years. Seropositivity at baseline was 11.5%. Geometric mean ferritin was 88.1 mg/L and mean plasma iron 0.82 mg/mL. Iron deficiency was generally low, with prevalence higher in females. In the longitudinal analysis, ferritin at time of vaccination was positively associated with Anti-S IgG Ab (b=0.004, 95% CI 0.002;0.007), and Anti-wildtype NAb (b=0.011, 95% CI 0.001;0.021), Anti-delta NAb (b=0.016, 95% CI 0.006;0.026), and Anti-omicron NAb neutralizing antibodies (b=0.013, 95% CI 0.003;0.024). Plasma iron results were generally not significant. Significantly lower half-life was observed in participants in the highest quartiles of ferritin concentrations, while no clear trend was observed for plasma iron. Conclusion: In this mostly iron replete cohort, ferritin at time of vaccination is was marginally associated with the vaccination-induced development of immune markers to SARS-CoV-2.



P-33 - THE CROSS-TALK BETWEEN IMMUNITY AND ERYTHROPOIESIS IN MALARIA-INDUCED ANEMIA: THE ROLE OF HFE

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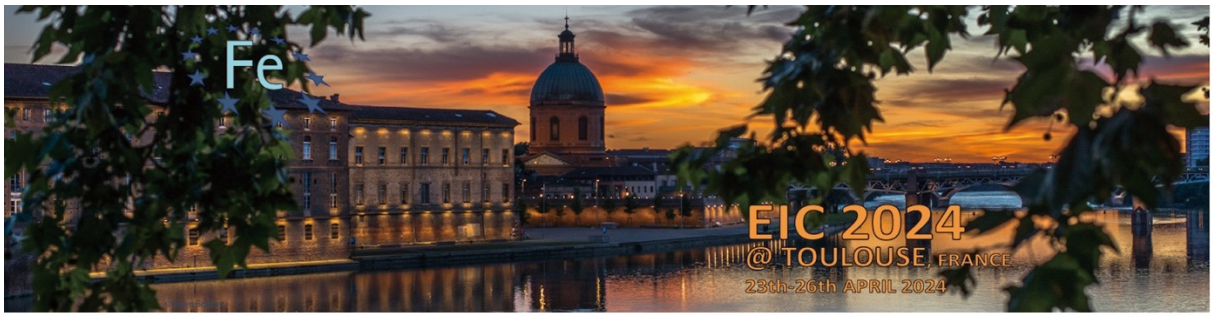
Severe malarial anemia (SMA) is a complication caused by Plasmodium infection. SMA is associated with an increased morbidity and mortality rate, due to an imbalanced cross-talk between immunity and erythropoiesis. Our study explores the impact of this interplay and the role of HFE on the development of SMA. Comparative analyses were conducted in wild-type and HFE-deficient mice, infected with Plasmodium chabaudi chabaudi, a strain causing high degrees of hemolysis. Although all mice survived the infection, the absence of HFE led animals to develop a lower parasitemia, when compared to wild-type. The anemic profile of deficient animals was improved. An increased bone marrow erythropoiesis was accompanied by a reduced red blood cells (RBCs) production in the spleen, known as stress erythropoiesis. This finding correlated to a robust activation of the immune system. An enhanced innate and adaptive immune response was found in mice deprived of HFE, in response to malaria. Specifically, a pronounced activation of CD4+/B cell axis was observed in relation to wild-type, in addition to a compensatory upregulation of MHC II. The exposure of HFE-deficient mice to a second Plasmodium infection, to mimic endemic occurrences, promoted a long-term immunity that influenced erythropoietin concentration and improved erythropoiesis. Changes in iron (Fe) metabolism were also compared upon infection, given the role of HFE as Fe sensor. Overall, our data demonstrated that the absence of HFE in mice prevented the severity of malaria by improving the cross-talk between immunity and erythropoiesis



P-34 - TRIGGERING THE SHIFT - SERUM IRON PROTEINS IN THE CROSSTALK BETWEEN IRON BIOLOGY AND IMMUNE RESPONSE

FONSECA Óscar [1,2], RAMOS Ana Sofia [1,3,4], COUTO Carolina [3,4], SILVA André [5], FERREIRA-DA-SILVA Frederico [1], SILVA Tânia [1,4], GOMES Maria Salomé [1,4], MOREIRA Ana Carolina [1,4,6]

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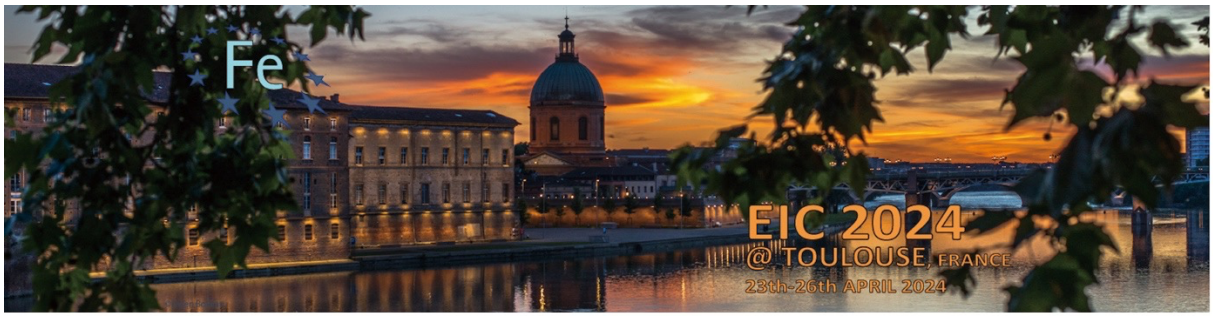
Serum iron is usually considered to be completely bound to transferrin in basal conditions. Our previous work using a mouse model of chronic infection revealed that a proportion of serum iron binds to proteins other than transferrin, as infection progresses. We termed this transition the “iron-shift” (IS). Our aim was to characterize the kinetics, protein profile and immune regulation of the IS, thus assessing its importance for iron re-distribution and host protection during infection. We infected C57BL/6 or genetically deficient mice with *Mycobacterium avium* and collected serum after 1 to 8 weeks. Serum proteins were separated using consecutive chromatographies, based on the proteins’ size (SEC), charge (IEX), and hydrophobicity (HIC). LC-MS was performed on the fractions with the highest iron content to identify putative iron-binding proteins. The IS was observed starting three weeks post-infection (PI) in wild-type, IFNg-, iNOS- and CCL2-deficient mice. Contrarily, the IS in TNFa knock-out mice was delayed and occurred only at four weeks PI, independently of the bacterial load. In all cases, the IS remained until the end of the experiments. Our preliminary results suggest that ferritin and/or haptoglobin might play important roles in this process. TNFa may cause alterations in serum iron binding during infection. The understanding of the crosstalk between iron biology and immune response may allow the development of new host-targeted therapies applicable to infections and other pathologies with iron disturbances.

This work was financed by Portuguese national funds through FCT – Fundação para a Ciência e Tecnologia, within the projects EXPL/BIA-BQM/1170/2021 and 2022.03635.PTDC, and PhD fellowship 2023.00714.BD to OF.



P-37 - Dysregulated iron metabolism shapes alveolar macrophage response to Streptococcus pneumoniae in copd

FAHERTY Lynne [1], HEARNE David [1], MUÑOZ-WOLF Natalia [1], HEALY Claire [1], CLOONAN Suzanne M. [1,2]
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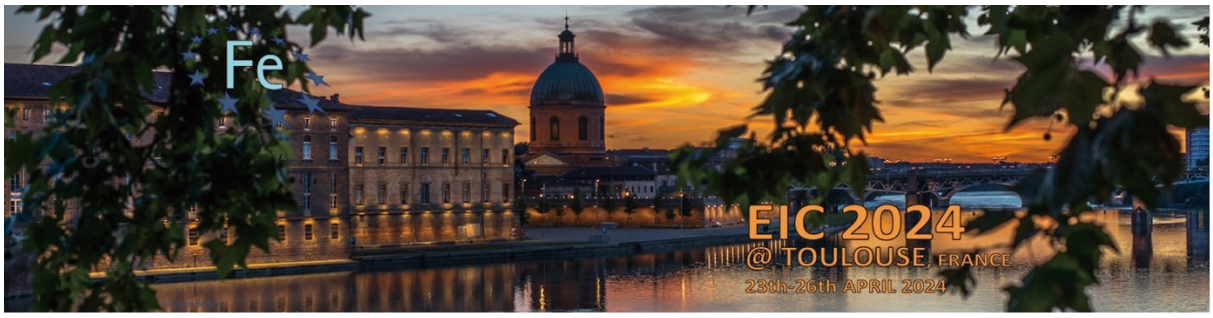
The necessity of iron as an enzymatic cofactor for metabolic reactions in both mammalian and microbial cells positions it as a key mediator of host-pathogen interactions, where the 'fight for iron' is a crucial determinant in the ability of pathogens to survive and replicate in the host. Systemically, macrophages are central in iron recycling and handling, but the effects of altered macrophage iron levels on infection outcomes are not fully understood. In chronic obstructive pulmonary disease (COPD), lung-resident alveolar macrophages (AMs) are iron loaded, and the abundance of iron in the extracellular milieu correlates with more frequent infection-driven exacerbation events. While such exacerbations are a large cause of morbidity and mortality in COPD, how macrophage iron loading affects host-pathogen interactions in the lung is poorly understood.

Here we show that *in vitro* and *ex vivo*, murine AMs subjected to experimental COPD have higher total and mitochondrial iron, confirmed through graphite furnace-atomic absorption spectrometry, confocal microscopy, and qPCR/western blotting of factors governing cellular iron metabolism. These "COPD" macrophages are more susceptible to infection with *Streptococcus pneumoniae*, a common respiratory pathogen in COPD, assessed by ELISA (IL-6, TNF-α and lipocalin-2) and enumeration of intracellular colony forming units (CFU). The sensitivity of *S. pneumoniae* intracellular replication to host iron levels was also demonstrated through CFU enumeration in murine AMs pre-treated with iron or iron chelators. These results highlight the role of dysfunctional iron metabolism in promoting bacterial virulence and suggest the targeted manipulation of macrophage iron as a novel therapeutic avenue in COPD.



P-38 - IRON-SULFUR CLUSTER BIOGENESIS AND HEME METABOLISM ARE ESSENTIAL FOR RESIDUAL DISEASE IN AML

SALAND Estelle [1], SARRY Jean-Emmanuel [1]
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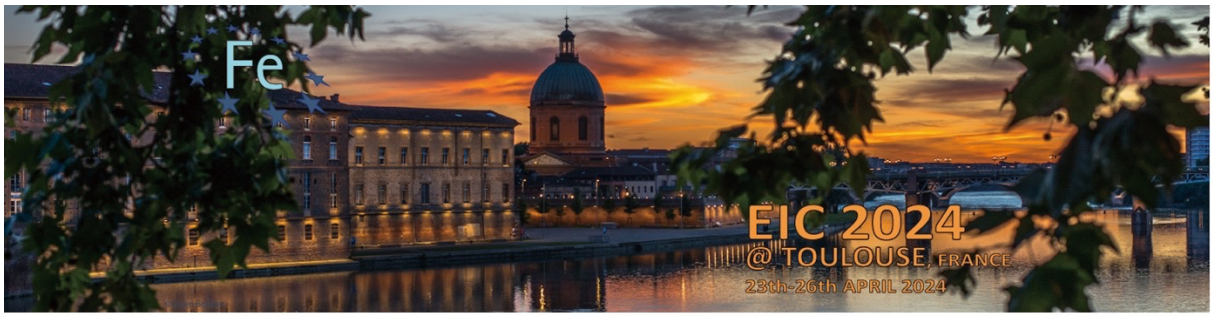
Mitochondrial activities, bioenergetics, dynamics and structure are crucial regulators of response and resistance to therapies in cancer including acute myeloid leukemia (AML). However, while cellular and systemic iron might contribute to mitochondrial functions and inflammation. the role of iron metabolism remains still largely unknown in the context of AML and/or drug resistance. Here, we first observed an increase in mitochondrial iron content in residual disease (RD) post-chemotherapy cytarabine in vitro and in vivo. Since mitochondrial iron takes a part for heme and iron-sulfur cluster (ISC) biogenesis, we noted that porphyrin biosynthesis and ISC machinery was stimulated in RD. Furthermore, activities of aconitase and ETCII, two mitochondrial ISC enzymes, were enhanced. Multi-omics analysis showed that Eltrombopag (ELT) was a potent modifying agent of mitochondrial iron balance, altering heme and ISC biosynthesis that led in turn to changes in energetic balance (OxPHOS, TCA cycle and carbon metabolism) and overcome AraC resistance in AML *in vitro* and *in vivo*. Importantly, we analyzed *ex vivo* response to doublet therapy AraC/ELT using primary AML samples and we observed that AML cells harboring TP53 mutations exhibited a good response to this doublet therapy. Accordingly, TP53-mutated AML models were more sensitive to AraC/ELT compared to TP53-wt AML models. Finally, bone marrow-associated macrophages were specifically modified and educated by TP53-driven tumors. Together, our results uncover heme/porphyrin and ISC biosynthetic pathways as targetable and critical pathways for maintaining an elevated OxPHOS in RD in an inflammatory macrophage-dependent manner.



P-39 - A ROLE FOR CD44 IN ERYHTROID IRON UPTAKE

BESSON-FOURNIER Céline [1], BERGER Mathieu [1], PLAYS Marina [2], MÜLLER Sebastian [2], PALIN Anais [1], LATOUR Chloé [1], COPPIN Hélène [1], ROTH Marie-Paule [1], RODRIGUEZ Raphaël [2], MEYNARD Delphine [1]
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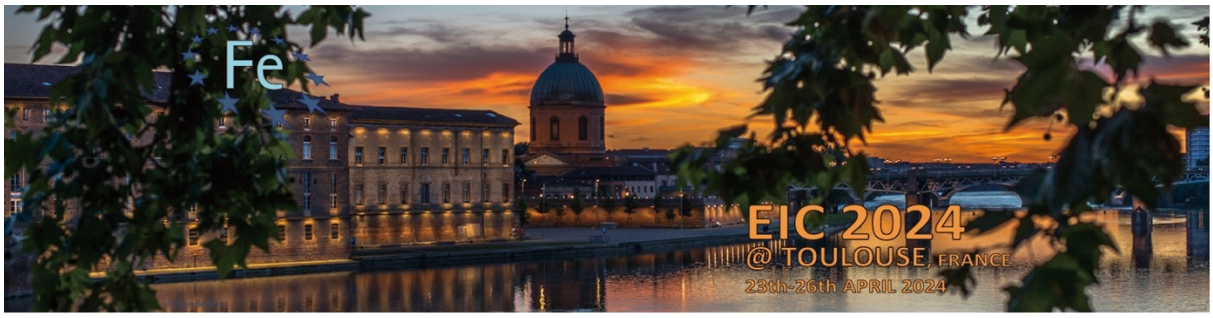
Red blood cells (RBC) transport oxygen to cells by binding oxygen to the iron cation of heme. Consequently, RBC production requires iron. Erythroid cells can acquire iron through the transferrin-transferrin receptor (TFR1) endocytosis cycle. In addition to this well-established pathway, studies suggest that erythroid cells may also utilize non-transferrin-bound iron (NTBI). Importantly, in the context of cancer and immune cells, CD44, a major receptor of hyaluronates, mediates endocytosis of iron-bound hyaluronates into the cells

In this study, we selectively deleted Tfr1 or Cd44 in erythroid cells using Tfr1 and Cd44 flox mice crossed with EpoR-Cre mice to assess their respective roles in iron uptake and erythroid maturation. We observed that Tfr1EpoR/+ mice are viable, albeit they exhibit anemia. In these mice, erythroid cells that are deleted for Tfr1 develop and mature normally, and importantly maintain the capacity to produce hemoglobin. By measuring endosomal Fe2+ with a fluorescent probe, we demonstrated that erythroid cells lacking Tfr1 have the same ability to acquire iron than WT cells, indicating the utilization of an alternative mechanism for iron acquisition.

To investigate if Cd44 could be involved in this mechanism, we deleted Cd44 in erythroid cells in mice. We observed a reduced production of RBC and hemoglobin in Cd44EpoR/+ males. Females lacking Cd44 in erythroid cells exhibited delayed recovery during erythroid stress induced by phenylhydrazine injection, along with a reduction in RBC production compared to WT mice. Taken together, these results suggest that both Tfr1 and Cd44 contribute to iron acquisition in erythroid cells.



P-40 - THE LINK OF FATTY ACID AND IRON METABOLISM IN THE CONTEXT OF TAMOXIFEN RESISTANCE

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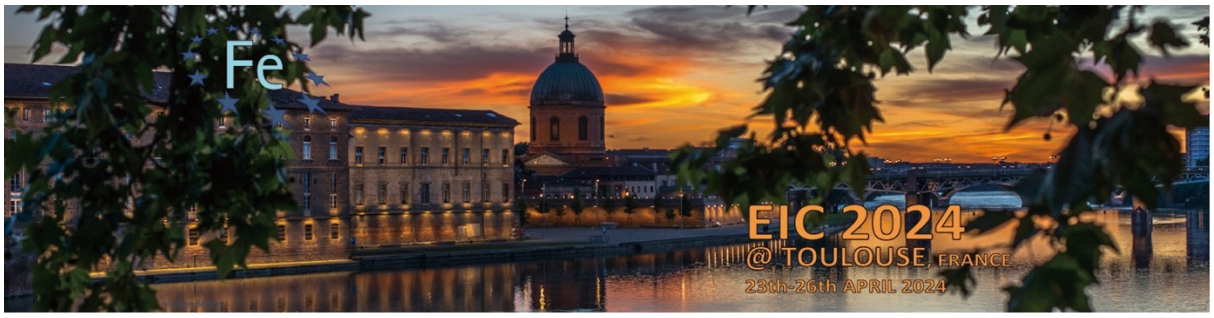
Tamoxifen resistance represents a major clinical problem during hormone therapy treatment. We have developed an in vitro model of tamoxifen-resistance in estrogen positive breast cancer cell lines MCF7 and T47D resistant to 5 µM tamoxifen (Tam5R cells). We have found that Tam5R cells show altered fatty acid metabolism, in particular in metabolism of glycerol phospholipids. Furthermore, we have identified that these cells have altered iron metabolism and asked whether there is an interplay between these phenomena. Our data showed a consistent up-regulation of enzymes involved in fatty acid synthesis such as ACSL1 and ACSS1 in Tam5R cells. At the same time, we have documented higher mitochondrial iron stores but lower iron utilization in these cells. Therefore, we have analyzed the response of ACSS1 and ACSL1 to iron deprivation and iron overload in wild-type and Tam5R cells. Interestingly, our data show that both ACSL1 and ACSS1 respond to iron deprivation and iron excess. ACSS1 seems to follow similar pattern as ACO2, a well-known iron-regulated mitochondrial enzyme, with IRE located at 5´ UTR. On the other hand, ACSL1 seems to show a pattern similar to TFR1, an iron-regulated transferrin receptor, whose mRNA stability is regulated by IRE present at 3´ UTR. Therefore, we propose that there is an interplay between fatty acid synthesis and iron metabolism which is disrupted in the context of tamoxifen resistance and may be regulated by the IRE/IRP system.
Funding: Funded by Czech Science Foundation project no. 23-06208S and MEYS Program EXCELES LX22NPO5102.



P-41 - HYDROXYCINNAMIC ACID DERIVATIVES AS BIFUNCTIONAL ANTIOXIDANTS AND IRON CHELATORS FOR PHOTOPROTECTION

ALBADAINEH Batool [1,2,3], GE Haobo [1,2,3], BLAGBROUGH Ian [1], LAOURI Io [1], BADURES Samaher [1], MA Yongmin [4,5], CILIBRIZZI Agostino [2,4], HIDER Robert [4], POURZAND Charareh [1,2,3]

[1] Life Sciences, University of bath, Bath, United Kingdom; [2] Centre for Therapeutic Innovation, University of Bath, Bath, United Kingdom; [3] Centre for Bioengineering and Biomedical Technologies, University of Bath, Bath, United Kingdom; [4] Institute of Pharmaceutical Science, King's College London, London, United Kingdom; [5] Institute of Advanced Studies, School of Pharmaceutical and Chemical Engineering, Taizhou University, Taizhou, China

Hydroxycinnamic acid derivatives such as trans-cinnamic acid (TCA), caffeic acid (CA), ferulic acid (FA), rosmarinic acid (RA) and chlorogenic acid (CHLA) are known to exhibit antioxidant activities in vitro and in vivo. Based on their chemical structures, they are also expected to exhibit iron chelating properties.[1] These compounds can be ideal skin photo-protectants against UVA component of sunlight, since UVA exerts its dual damaging effect in the skin by both generating reactive oxygen species (ROS) and promoting the release of harmful labile iron (LI), that acts as a catalyst to exacerbate the ROS-mediated damage.[2] We first evaluated the intracellular LI levels before and after UVA irradiation of human skin fibroblasts treated (or not) with TCA, CA, FA, RA and CHLA using custom-made fluorescent iron-selective sensors[3,4] and then corroborated these with the extent of protection against UVA-induced cell damage with MTT assay. Among all compounds studied, TCA provided the most promising photoprotection against UVA in skin cells that corroborated with its strong ability to decrease both the basal and UVA-induced increase in intracellular LI.This is the first study demonstrating the dual function of hydroxycinnamic derivates notably TCA against UVA damage in skin cells.

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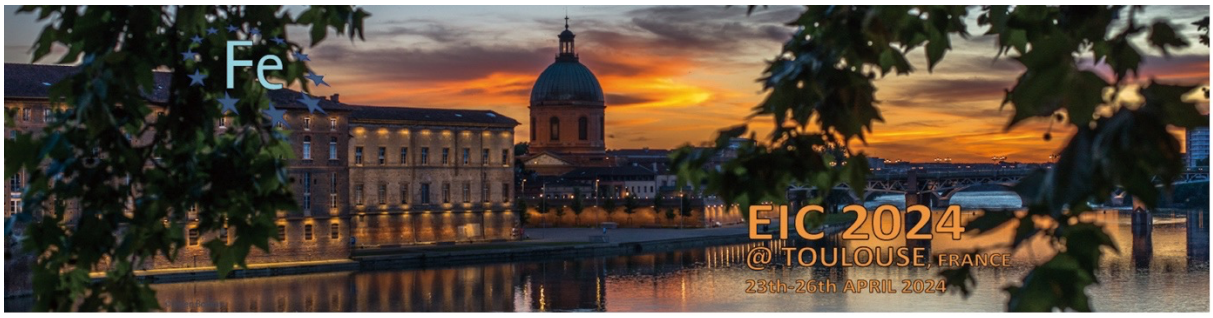
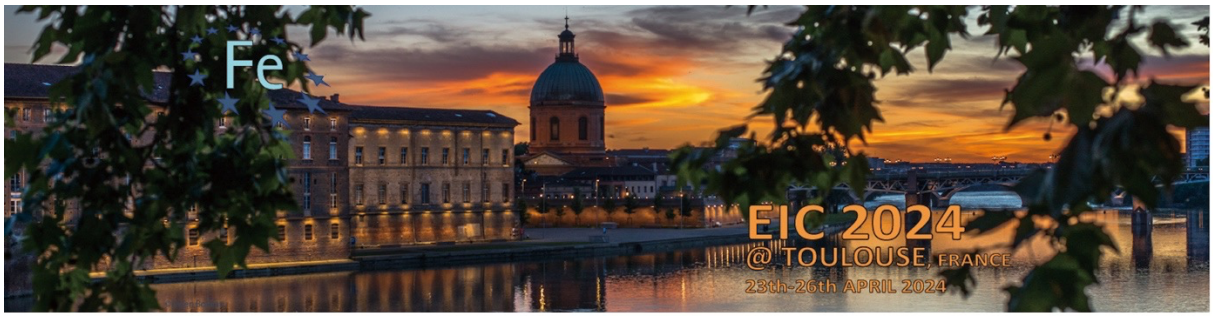


P-42 - THE UNEXPECTED LINK BETWEEN IRON REGULATION AND HYPOSPERMATOGENESIS

HARRER Aileen [1], GHATPANDE Niraj [2], GRIMALDINI Tiziana [1], FIETZ Daniela [3], PLEUGER Christiane [1], FIJAK Monika [1], FÖPPEL Dankward [1], RYNIO Lennart [1], WUDY Stefan A. [4], GUTTMANN-RAVIV Noga [2], BHUSHAN Sudhanshu [1], MEYRON-HOLTZ Esther G. [2], MEINHARDT Andreas [1]

[1] Institute of Anatomy and Cell Biology, Unit of Reproductive Biology, Justus-Liebig-University of Giessen, Giessen, Germany; [2] Faculty of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa, Israel; [3] Institute for Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University of Giessen, Giessen, Germany; [4] Steroid Research and Mass Spectrometry Unit, Pediatric Endocrinology and Diabetology, Center of Child and Adolescent Medicine, Justus-Liebig-University of Giessen, Giessen, Germany

Imbalances in testicular iron levels have been associated with compromised sperm production and male infertility. Iron regulatory proteins (IRP) 1 and 2 plays cruicial roles in cellular in regulating the iron regulation. In the present study, we investigated the role of IRPs on archetypical testicular functions, i.e. spermatogenesis and steroidogenesis, by employing *Irp*1 (*Irp*1$^{-/-}$) and *Irp*2 (*Irp*2$^{-/-}$) defficient mice. In *Irp*1$^{-/-}$ testis, hypospermatogenesis was observed histologically exemplified by a clear reduction in the number of elongated spermatids and daily sperm production compared to wild-type (WT) and *Irp*2$^{-/-}$ mice. In addition, stage 8 of spermatogenesis (sperm release) was reduced in testicular cross-sections of *Irp*1$^{-/-}$ testis. Furthermore, flow cytometry analysis of germ cells derived from WT and *Irp*1$^{-/-}$ showed that beside elongated spermatids all germ cell types were reduced in numbers, which was further confirmed by immunofluorescence. Notably, hypospermatogenesis worsened with age despite unchanged intratesticular iron levels. Although IRP1 was mainly localized to Sertoli cells in human biopsies and mouse testis cross-sections, Sertoli cell numbers remained similar in *Irp*1$^{-/-}$ compared to WT testes. Levels of androgens (e.g. testosterone and corticosteron) as examined using mass spectrometry were unchanged across the investigated genotypes. Our initial results suggest impaired meiotic induction as a possible basis of hypospermatogenesis. Altogether, this study uncovers a novel role for IRP1 in maintaining germ cell survival and spermatogenesis, independent of iron regulation. These findings help to understand the complex interplay between iron regulation and critical testicular functions.



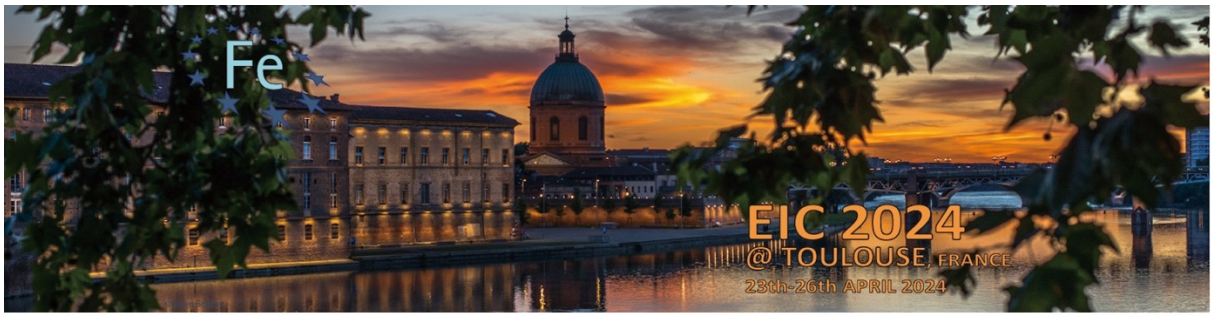
P-43- IRON REGULATION IN SKELETAL MUSCLE DIFFERS DEPENDING ON MUSCLE WASTING AND IRON OVERLOAD CONDITIONS

AUFFRET Maëlys [1], ORFILA Luz [1], HOREAU Mathieu [1,2], ROPERT Martine [2], FREYSSENET Damien [3], LORÉAL Olivier [2], RÉBILLARD Amélie [1], DERBRÉ Frédéric [1]

[1] Laboratory "Movement Sport and Health Sciences" (M2S), EA7470, University Rennes 2, Rennes, France; [2] INSERM, University of Rennes, INRAE, AEM2 platform, Nutrition Metabolisms and Cancer (NuMeCan) institute, Rennes, France; [3] Inter-University Laboratory of Human Movement Biology EA 7424, Univ Lyon, University Jean Monnet Saint-Etienne, Saint-Priest-en-Jarez, France

Iron metabolism is crucial in various physiological processes, especially for maintaining muscle function. Previous studies have reported relationships between muscle wasting and iron overload, but the underlying mechanisms in these physiopathological conditions are incompletely characterised. The aim of this study was to characterize in vivo the impact of muscle wasting and secondary iron overload on iron regulatory proteins in skeletal muscle. For this purpose, we used murine models supplemented with iron-dextran (1g/kg), or exhibiting muscle atrophy induced by cancer cachexia (ApcMin/+) or simulated microgravity (hindlimb unloading). Histological and biomolecular analyses were performed on glycolytic skeletal muscle (i.e. quadriceps or gastrocnemius).

Despite intramuscular iron overload, iron-dextran mice do not exhibit muscle mass loss and do not seem to actively facilitate iron efflux, as indicated by the lack of changes of ferroportin and FLVCR1 proteins. Regarding atrophy models, muscle weight loss in unloaded animals is associated with iron accumulation in gastrocnemius, while muscle wasting do not induce iron excess in quadriceps in ApcMin/+mice. However, in both cases, the atrophied skeletal muscles seem to favor iron sequestration by limiting export through ferroportin. Whatever the experimental model, neither myoglobin nor FLVCR1 are modulated in skeletal muscle, suggesting that heme iron might not be specifically regulated under muscle wasting or iron overload conditions. Muscle iron metabolism appears to respond differently depending on the cause of muscle iron overload (i.e. systemic iron overload, disuse or inflammatory origin of atrophy). These results suggest a complex interplay between atrophy pathways and iron homeostasis with skeletal muscle tissue.



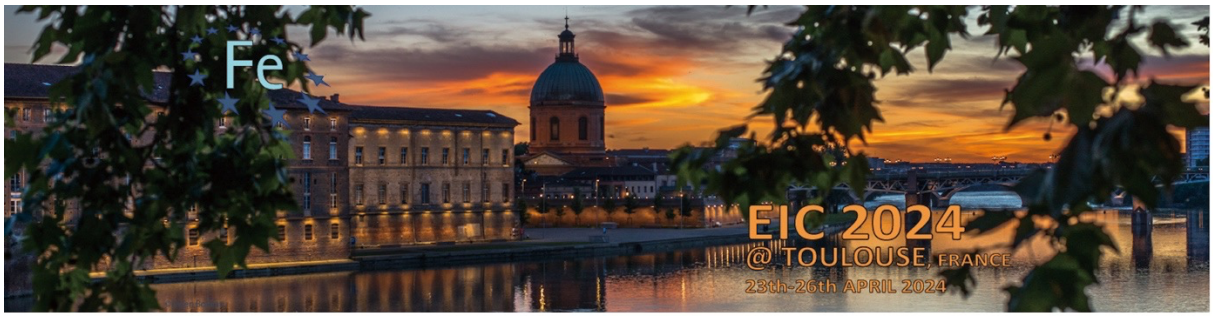
P-44 - UNRAVELING HFE-INTERACTION PARTNERS AND SIGNALLING: IMPACT ON CELLULAR IRON HOMEOSTASIS

STEGEMANN Gaby [1], BLANS Colin [1], VERKUIJLEN Paul [1], VAN BRUGGEN Robin [1], SWINKELS Dorine [1,2], MATLUNG Hanke [1]

[1] Department of Molecular Hematology, Sanquin Research and Landsteiner Laboratory, Amsterdam University Medical Centre (AUMC), University of Amsterdam, Amsterdam, Netherlands; [2] Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, Netherlands

Tissue iron overload is the most common side effect after RBC transfusion. As the human body has no regulatory mechanisms to excrete iron, long-term transfusion inevitably produces the clinical problem of iron overload. The patient's underlying disease determines iron distribution among cell types. Overall, iron accumulation in parenchymal cells is considered more toxic than that in cells of the reticuloendothelial system. We hypothesize that sensitivity of a cell to iron-induced toxicity is determined by the net outcome of transferrin-bound iron (TBI) and non-transferrin-bound iron (NTBI) uptake rates, capacity of iron export, regulation of iron deposition into ferritin and capacity of the cell to initiate a proper antioxidant defense. The hereditary hemochromatosis protein (HFE) is implicated to have a regulatory role in a number of these processes, including import of TBI and NTBI through an interplay with TfR2, TfR1 and NTBI-transporter ZIP14. HFE also inhibits iron release from macrophages either by systemic control of ferroportin via induction of hepatic hepcidin production and/or by direct cell-specific regulation of its expression.

We aim to identify HFE-dependent regulatory pathways involved in cellular iron homeostasis, and how these cell-specific and/or systemic regulatory pathways contribute to transfusion and iron overload-related toxicity. So far, we have successfully generated hepatocyte cell lines overexpressing HFE tagged with miniturbo-ID for biotin proximity labeling. Currently, we are validating our cell systems and plan to identify downstream signaling and complex partners of HFE under conditions of iron overload and relate the identified proteins to their role in iron homeostasis and toxicity.



P-45 - IRON LOADING IN ALVEOLAR MACROPHAGES ALTERS ALVEOLAR TYPE II EPITHELIAL CELL FUNCTION IN COPD

WUGK Sophia [1], CLOONAN Suzanne [1,2]
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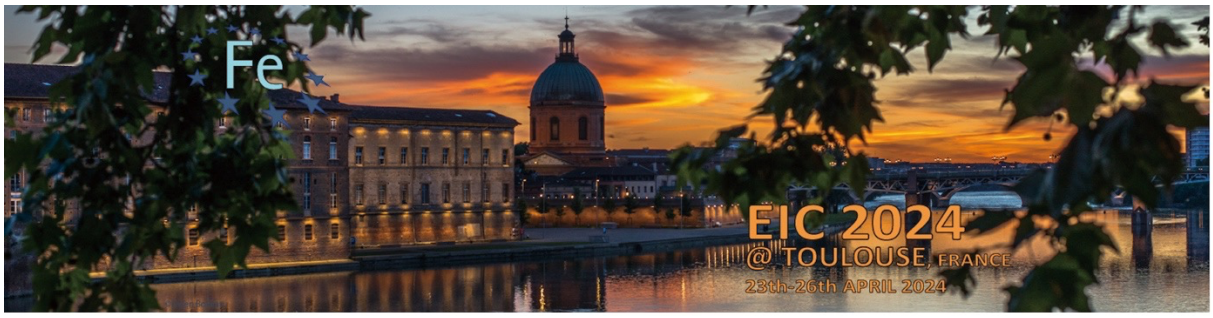
Background: Continuous crosstalk between alveolar macrophages (AMs) and the alveolar epithelium is essential to maintaining alveolar health. While cellular iron loading in AMs has clearly been characterized in chronic obstructive pulmonary disease (COPD), little is known about whether these iron-loaded AMs contribute to alveolar epithelial cell (AEC) dysfunction and tissue damage in COPD pathophysiology.

Objectives/Hypothesis: This study aimed to show the effects of iron loading in AMs on alveolar type II epithelial cell (AEC2) cell function in an in vitro model of COPD using murine foetal liver-derived alveolar macrophages (FLAMs).

Materials and Methods: Iron loading in FLAMs was achieved by treatment with 26.5 ng/ml ferric ammonium citrate, 10 µM ferrous lactate, or 5% cigarette smoke extract for 24h. Treatments were washed away, and cells were allowed to secrete immunometabolic mediators into media for 6h. MLE-12 cells (an AEC2 cell line) were incubated with conditioned medium of FLAMs or cocultured with pre-treated FLAMs, spatially separated by trans-well inserts, for 24h. Changes in MLE-12 viability, function, and cellular iron metabolism were assessed by alamarBlue assay, immunoblotting, and RT-qPCR. Cellular iron contents were measured by graphite furnace absorption spectroscopy.

Results: Iron loading in FLAMs did not significantly affect MLE-12 viability, but altered MLE-12 cell identity markers as well as their function in vitro.

Conclusion: Dysregulated crosstalk between iron-loaded AMs and AEC2s may contribute to COPD pathophysiology by inducing AEC2 dysfunction and tissue damage.



P-46 - Exploring Iron Dynamics in Alveolar Type II Epithelial Cells: Implications in Chronic Lung Diseases

KENNY Sarah [1], HUANG Ziling [2,3], KIM Kihwan [2], CAPILI Allyson [4], CARPENTER Susan [4], WARD Diane [5], CLOONAN Suzanne [1,2]

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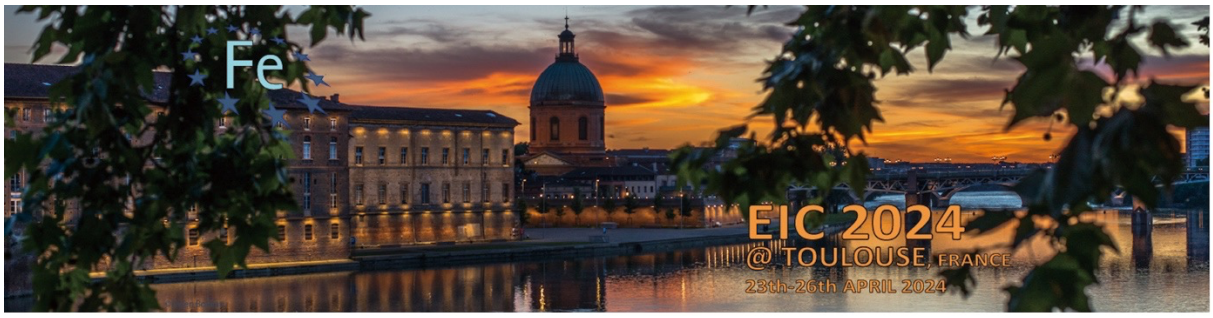
Alveolar type II epithelial (AT2) cells lie at the centre of alveolar maintenance and function. These metabolically active cells primarily function to produce and secrete surfactant, an essential phospholipid-rich compound that maintains surface tension, preventing alveolar collapse. Accumulating evidence illustrates a role for AT2 cell dysfunction and senescence in several chronic lung diseases, including Idiopathic Pulmonary Fibrosis (IPF). Interestingly, disrupted iron homeostasis is implicated in IPF pathogenesis, yet the interplay between iron levels and dysfunctional AT2 cells is poorly understood. Here we show manipulating iron levels in AT2 cells alters their function both in vitro and in vivo. Iron-loaded MLE12 cells, a murine AT2 cell line, have a reduced wound healing capacity, and increased secretion of surfactant protein C (SftpC) coupled with a decrease in surfactant lipid uptake. Decreased intracellular iron levels in MLE12 cells have varying effects on function depending on the mechanism by which iron is reduced. Deferoxamine (DFX) chelation, induces the upregulation of senescent and pro-fibrotic markers with the opposite being observed for the intracellular iron chelator deferiprone (DFP). Depletion of mitochondrial iron transporters in AT2 cells both in vitro and in vivo results in a similar senescent/fibrotic phenotype. Using a lung injury and fibrosis model, namely bleomycin insult, drives iron-overload, cellular senescence, and decreased SFTPC levels in vitro. However, isolated AT2 cells, from bleomycin-exposed mice, present with decreased intracellular iron levels. Overall, these findings underscore the significant involvement of iron in AT2 biology, emphasizing the complexity of how iron dyshomeostasis contributes to AT2 dysfunction in disease.



P-47 - THE SIRES (SEARCHING FOR IRON RESPONSIVE ELEMENTS) SOFTWARE IS BACK

SUAREZ-QUINTANA Clara [1,2], CHOROSTECKI Uciel [2], **SANCHEZ Mayka [1]**

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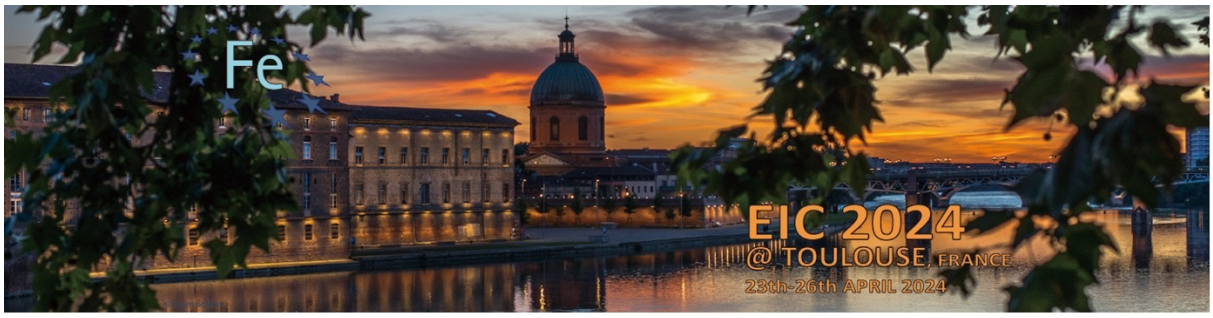
The IRP/IRE regulatory system, pivotal in post-transcriptional cellular iron control, comprises iron-responsive elements (IREs) – mRNA cis-acting motifs interacting with iron regulatory proteins (IRP1 and IRP2). Despite its early characterization (Hentze MW, et al. Science. 1987), this regulatory system remains a focal point of interest across various biological disciplines.

In this context, we present the latest version of the Searching for Iron Elements web server, SIREs version 3.0, designed for predicting IREs based on sequence input. This update introduces two user-friendly modes that allow easy queries from NCBI and Ensembl using an accession number, along with an expanded set of apical loop motifs for efficient screening. SIREs 3.0 also provides the IRE location within transcripts and details on distance to RNA cap, start and end codons, all of them features that have been proposed to affect the functionality of putative IREs. Additionally, this version introduces a visual reference to aid in comparing predicted scores and free energies with canonical IREs, and a refined score system that accounts for fine-grained sequence and structural considerations based on experimental data.

To ensure continued maintenance, future updates and integration with other services, SIREs 3.0 has been migrated to Python. Finally, a renewed web interface has been implemented to provide a friendly user experience. With these improvements, SIREs 3.0 is set to offer more comprehensive predictions, reaching a broader audience and providing valuable support to the iron community. SIREs 3.0 is accessible at http://sires-webserver.eu Supported by grants PID2021-122436OB-I00 from MCIN/AEI /10.13039/501100011033 to MS



P-48 - EXPLORING THE ROLE OF TRANSFERRIN RECEPTOR 1 IN BONE HOMEOSTASIS

PASSIN Vanessa [1], LEDESMA-COLUNGA Maria G. [1], HOFBAUER Lorenz C. [1], RAUNER Martina [1]
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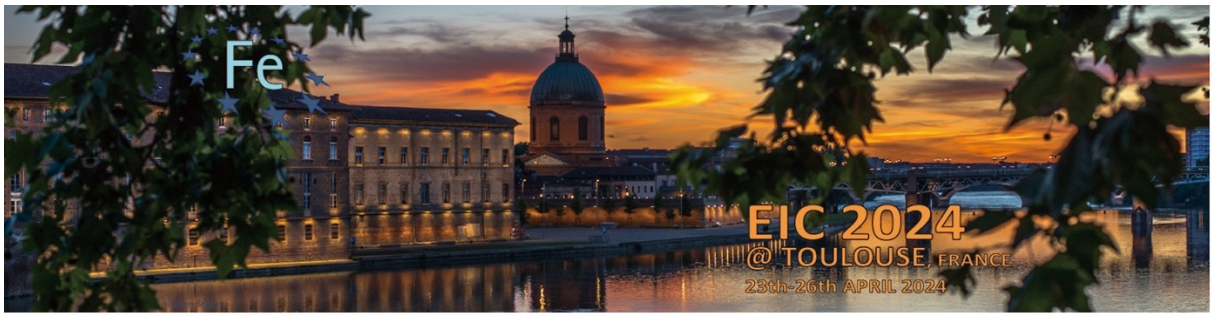
Bone health relies on balanced iron levels, with both iron overload and deficiency contributing to increased fracture risk. However, the mechanisms of iron acquisition in bone cells remain largely unknown. In this study, we investigated the significance of transferrin receptor 1 (Tfr1) for the cellular function in osteoblasts and osteoclasts. Twelve-week-old male and female $Tfr1^{fl/fl}$;Osx:Cre+ (osteoblasts) and $Tfr1^{fl/fl}$;LysM:Cre+ (osteoclasts) conditional knockout mice, along with their littermate controls, were used to assess bone microarchitecture as well as systemic bone turnover markers PINP and TRAcP5b (microCT, histomorphometry, ELISA). Bone marrow-derived cells from these mice were cultured *in vitro* to assess cellular iron status, differentiation, and function (qPCR, Alizarin Red S, TRAP staining, western blot).

$Tfr1^{fl/fl}$;Osx:Cre+ males showed an increased BV/TV at the lumbar spine [1.2-fold; $p<0.01$] with a decreased bone formation rate [2.2-fold; $p<0.05$] and lower levels of serum bone turnover markers [PINP: 1.3-fold; $p<0.01$, TRAcP5b: 1.5-fold; $p<0.01$]. Similarly, females displayed a higher femoral BV/TV [1.5-fold; $p<0.01$]. Tfr1-deficient osteoblasts showed decreased ferritin protein levels [1.3-fold; $p<0.05$] but no changes in mRNA levels of osteoblast-specific genes nor in their mineralization capacity. Conversely, Tfr1 deficiency in osteoclast precursors did not alter the bone phenotype in mice nor the iron status and differentiation of osteoclasts *ex vivo*.

Taken together, Tfr1 might be important for iron uptake in osteoblasts, ensuring proper bone turnover, while its deletion in osteoclasts has no impact on bone microarchitecture. These results emphasize diverse iron acquisition strategies used by various cell types to maintain cellular iron homeostasis.



P-49 - BRAIN IRON AND RISK OF DEMENTIA AND PARKINSON'S DISEASE: A GENETIC ANALYSIS IN THE UK BIOBANK COHORT

CASANOVA Francesco [1], TIAN Qu [2], WILLIAMSON Daniel [3], QIAN Yong [2], ZWEIBAUM David [2], DING Jun [2], ATKINS Janice [1], MELTZER David [1], FERRUCCI Luigi [2], PILLING Luke [1]

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Introduction

Iron overload is observed in neurodegenerative diseases, especially Alzheimer's disease (AD) and Parkinson's disease (PD). Whether brain iron deposition is causal or secondary to the neurodegenerative processes is unclear.

Methods

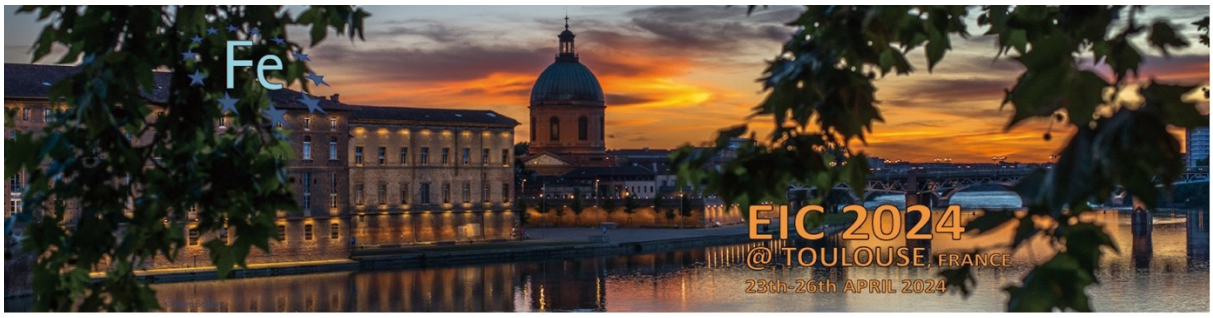
We analysed UK Biobank participants of European genetic ancestry (N=451,231), including 39,533 with brain MRI data. We performed genome-wide association studies (GWAS) on brain iron estimated by R2* and quantitative susceptibility mapping (QSM) in subcortical regions of accumbens, amygdala, caudate, hippocampus, pallidum, putamen, substantia nigra and thalamus. We used Mendelian randomisation to examine the causal effect of brain iron on AD, non-AD, PD and grey matter volumes as well as exploring if genetic liability to AD and PD was associated with increased R2* and QSM.

Results

In GWAS, we replicated 83% of previously reported genetic loci for R2* and QSM and identified additional 174 loci for R2* or QSM across all brain regions. Genetically predicted higher R2* and QSM in putamen and thalamus were associated with increased risk of non-AD dementia (Odds Ratio(OR)-QSM~putamen 1.15(1.05;1.25), p=0.001) and lower corresponding grey matter volumes but was not associated with AD risk (p>0.05). Genetic liability to AD was associated with QSM in the amygdala only. Genetically predicted higher iron in the caudate, putamen, and substantia nigra were associated with an increased risk of PD (OR-QSM~substantia-nigra 1.21(1.07;1.37), p=0.003). Genetic liability to PD was not associated with R2* or QSM.

Conclusion

We found genetic evidence of a likely causal effect of higher iron deposition in specific subcortical brain regions for non-Alzheimer's dementia and Parkinson's disease.

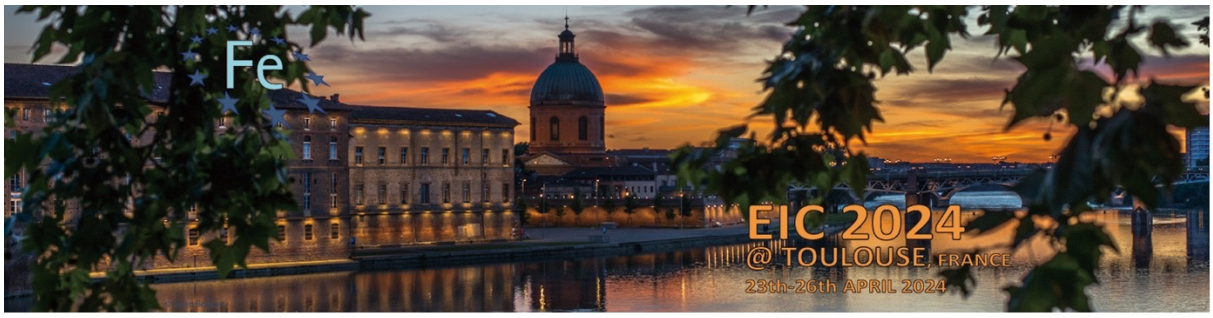


P-50 - A POTENTIAL ROLE OF PHOSPHORYLATION IN CERULOPLASMIN STABILITY AND ACTIVITY

MAGHERINI Giulio [1,2], DENARDO Andrea [2], ZURLO Giada [2], ZILIOTTO Nicole [2,3], NARDINI Ilaria [2], SANTUCCI Annalisa [1], CARICASOLE Andrea [2]

[1] University of Siena, Siena, Italy; [2] Kedrion Biopharma, Lucca, Italy; [3] University of Pisa, Pisa, Italy

Aceruloplasminemia (ACP) is a rare disease caused by mutations in ceruloplasmin (CP) gene, resulting in insufficient ferrous iron oxidation and accumulation in various organs, including the brain. Accordingly, neurodegeneration is the main hallmark for ACP, together with microcytic anemia and high serum ferritin levels. Moreover, CP is neuroprotective by inhibiting the neuroinflammation mediator myeloperoxidase (MPO) (Kono and Miyajima 2006). Previous work demonstrated that plasma-derived CP administration ameliorated neurodegeneration in an ACP mouse model (Zanardi, Conti et al. 2018). In this work, we sought to characterize effects of CP phosphorylation on its stability and activity (Bielli, Bellenchi et al. 2001, Hellman, Kono et al. 2002). We propose that steric hindrance of the negatively charged phosphate groups close to protease cleavage sites may hamper the protease docking, protecting CP against degradation. Inactivating mutations of two trypsin cleavage sites on CP, R450 and K906 (2APC mutation), allowed us to focus solely on the R720 site, that is in proximity to S722 and S725 residues which are subject of phosphorylation (Bielli, Bellenchi et al. 2001, Tagliabracci, Wiley et al. 2015). Using a combination of phospho-mimetic and phospho-abrogative mutations and kinase overexpression we demonstrated that phosphorylation at these residues influences the ability of trypsin to cleave at R720. As other serine/threonine phosphorylation sites are in proximity of known protease cleavage sites in CP, we will apply the same approach to define the role of phosphorylation on CP proteolytic cleavage with the aim to develop more stable CP variants as improved protein replacement therapeutics for ACP.



P-51 - MUSCULAR ADAPTATIONS TO IRON DEFICIENCY ANEMIA AND THE REVERSIBILITY AFTER INTRAVENOUS IRON

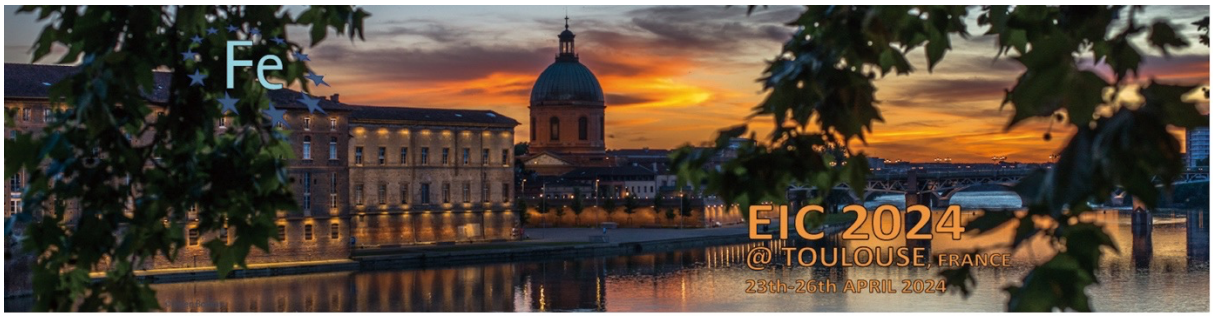
PERTLER Elke [1,2], WAGNER Sonja [1,2], OBHOLZER Laura [2], SCHÄFER Benedikt [2], PANZER Marlene [1,2], OBERACHER Herbert [3], SARG Bettina [4], FASERL Klaus [4], TILG Herbert [2], ZOLLER Heinz [1,2]

[1] Christian Doppler Laboratory for Iron and Phosphate Biology, Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria; [2] Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria; [3] Institute of Legal Medicine and Core Facility of Metabolomics, Medical University of Innsbruck, Innsbruck, Austria; [4] Division of Medical Biochemistry, Protein Core Facility, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

Background: Fatigue is a cardinal symptom of iron deficiency anemia (IDA) that can rapidly improve after intravenous (IV) iron. Iron is essential for mitochondrial function and energy production. Hormonal control, training, substrate- and oxygen availability determine which nutrients are used for energy production in muscles. We aimed to investigate the effects of IDA on muscle cells and the reversibility of adaptive changes after IV iron treatment. **Methods:** IDA was induced in three-week-old C57Bl/6 mice with dietary iron deficiency and controlled phlebotomy. Animals were injected with three different IV iron formulations. Seven days after IV injection, full blood count, gastrocnemius-, soleus-, plantaris-muscle, the diaphragm and the myocardium were analyzed. Gene expression was quantified by RT-qPCR. Metabolites and protein abundances were measured by untargeted LC-MS/MS. Fiber types were visualized by immunofluorescence staining.

Results: In IDA, energy production in skeletal muscle switched to anaerobic metabolism. Furthermore, oxidative fibers were significantly smaller, which correlated with hemoglobin concentration. Myocardial glucose metabolism was upregulated in IDA. However, pyruvate dehydrogenase was inactivated, resulting in increased lactate production. Respiratory chain and energy production were downregulated in both organs. IV iron treatment reversed most of the alterations caused by IDA, but the time course of the reversibility differed between the IV iron formulation tested.

Conclusion: The metabolic profile and preferred energy source differ between skeletal muscle and the myocardium in our IDA model. The structural and biochemical remodeling in skeletal muscle of IDA animals can be differentially reversed by IV irons. Signals controlling these adaptations will be further investigated.

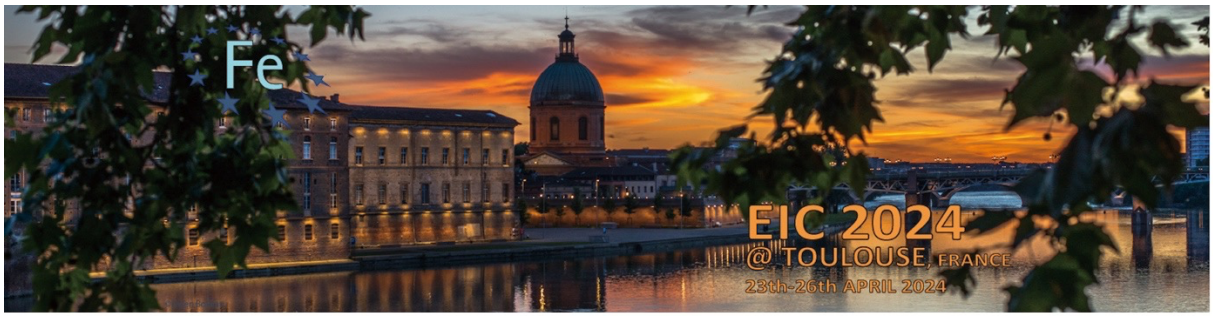


P-52 - THE FAILING HEART SHOWS A PATTERN OF IRON ACCUMULATION IN FIBROTIC AREAS

MERTENS Christina [1,2,3], SAADATMAND Alireza [3,4], DEWENTER Matthias [3,4], ANTONOVICI Ana [8], SCHENZ Judith [8], WEIGERT Andreas [5], MÖBIUS Andreas [6], KARCK Matthias [6], WARNECKE Gregor [6], WEIGAND Markus A [8], FISCHER Dania [8], BACKS Johannes [3,4,7], MUCKENTHALER Martina U [1,2,3,7]

[1] Department of Pediatric Hematology, Oncology and Immunology, Heidelberg University Hospital, Heidelberg, Germany; [2] Center for Translational Biomedical Iron Research, Heidelberg, Germany; [3] German Centre for Cardiovascular Research (DZHK), partner site Heidelberg/Mannheim, Germany; [4] Institute of Experimental Cardiology, Heidelberg, Germany; [5] Institute of Biochemistry I, Faculty of Medicine, Goethe-University , Frankfurt am Main, Germany; [6] Department of Cardiothoracic Surgery, Heidelberg University Hospital, , Heidelberg, Germany; [7] Molecular Medicine Partnership Unit, EMBL , Heidelberg, Germany; [8] Department of Anesthesiology, Heidelberg University Hospital, Heidelberg, Germany

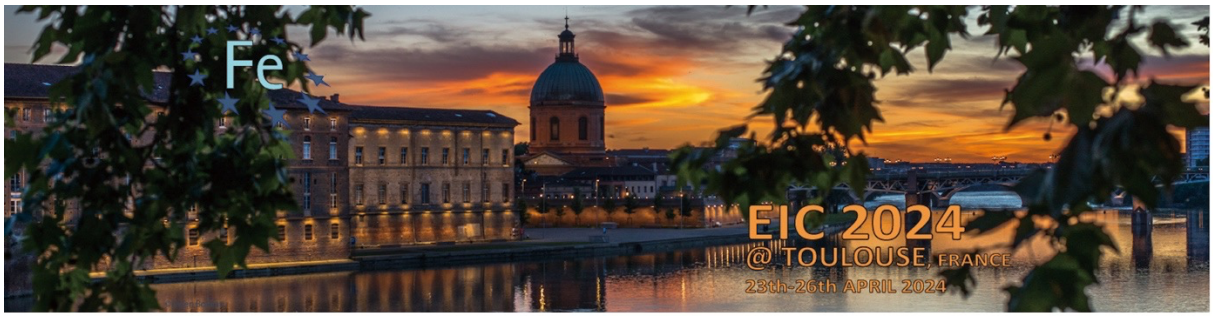
Both iron-overload and iron-deficiency negatively impact cardiac health. We aim to understand how changes in cardiac iron-availability affect cardiac iron distribution and cardiac function in steady-state and conditions of heart-failure. As a murine disease model of heart-failure, we apply the O-ring-aortic-banding (ORAB) in twelve-week-old C57BL6/N wildtype mice maintained on diets with low- or high-iron content. As expected, a decreased dietary iron content causes iron-deficiency with a mild anemic phenotype, while dietary iron supplementation led to iron accumulation in the liver, and increased serum ferritin values. Interestingly, cardiac iron levels only showed mild responses to the dietary iron manipulations. Furthermore, we show that independent of the systemic iron content, ORAB mice develop heart-failure as shown by a comparable reduction in ejection-fraction and a similar increase in left-ventricular mass. Our data suggest that alterations in steady-state iron-availability before the ORAB-surgery do not significantly influence heart-failure outcome and that rather iron redistribution in the heart may affect cardiac damage and regeneration. To understand whether iron accumulation in macrophages induces phenotypic changes, we applied multiplex-immunohistochemistry. Indeed, we found in wildtype mice after the ORAB-surgery iron accumulation in the vicinity of fibrotic areas. Importantly, the same pattern of iron accumulation was observed in human cardiac biopsies from patients undergoing open-heart-aortic-valve replacement. Our findings highlight the need for an improved understanding how cardiac iron distribution affects cardiomyocyte function. This knowledge will be fundamental to improve our understanding of the iron metabolism in the heart and aid the development of novel therapeutics maintaining iron homeostasis during heart-failure.



P-53 - MYOCARDIAL LABILE IRON DYNAMICS IN ACUTE AND CHRONIC IRON OVERLOAD

LIM Dillon Yee [1], BALL Charlotte [1,2], KABIR Syeeda Nashitha [1], VERA-AVILES Mayra [1], LAKHAL-LITTLETON Samira [1]
[1] Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom; [2] Department of Chemistry, University of Oxford, Oxford, United Kingdom

The labile iron pool (LIP) typically constitutes a small proportion of total cellular iron, but is responsible for cytotoxicity when in excess. The heart is particularly prone to taking up excess iron from the circulation, however changes in cardiac cellular LIP in response to acute or chronic iron loading are unexplored. We used iron-caged luciferin (ICL-1), a novel luminescent reporter of the LIP in luciferase transgenic mice to determine how cardiac LIP changes in two conditions – firstly, treatment with patient-equivalent dose of ferric carboxymaltose (FCM, Ferinject®), a third-generation intravenous iron therapy, and secondly, an inducible haemochromatosis model giving progressive iron overload. Haemochromatosis was not associated with significant changes in cardiac LIP despite hepatic iron loading. In contrast, IV iron treatment significantly increased cardiac LIP just 1 hour after administration, in the absence of hepatic iron elevation but associated with significant increases in serum iron levels. Additionally, we found evidence to suggest preferential right atrial uptake after IV iron treatment. Chamber-specific differences in expression of genes regulating iron homeostasis were identified which could account for this. Indeed, right atrial tissue displayed significantly greater expression of hepcidin and significantly lower expression of ferritin when compared to tissue from other chambers. These results indicate that FCM acutely elevates cardiac LIP. Further studies are warranted to examine the functional effects of FCM on the heart, and to explore intracardiac differences in iron homeostasis, all of which will are relevant to understanding the response to and safety of FCM in patients.

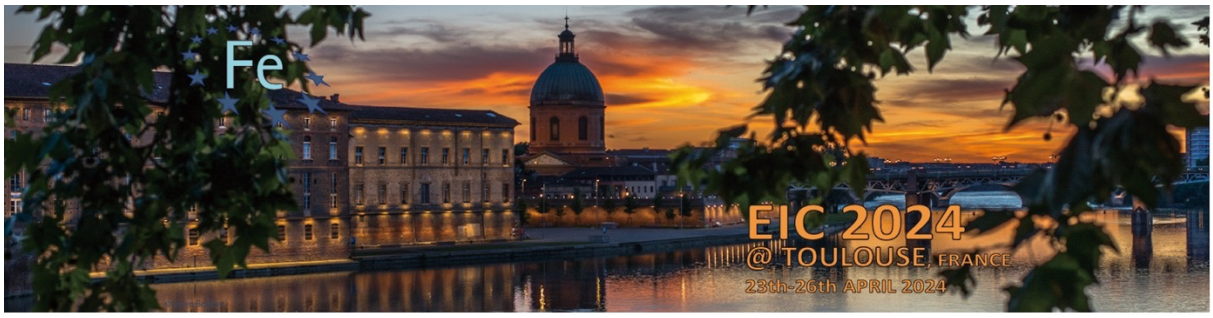


P-54 - IRON OVERLOAD IN HUMAN IPS CARDIOMYOCYTES

PEISCHARD Stefan [1], KASTL Philipp [2], KLINGMUELLER Ursula [2], SEEBOHM Nathalie [1], STEINBICKER Andrea [3], RAUNER Martina [4], **SEEBOHM Guiscard [1]**

[1] Institute for Genetics of Heart Diseases (IfGH), Department of Cardiovascular Medicine, University Hospital Münster, Münster, Germany; [2] Division Systems Biology of Signal Transduction, German Cancer Research Center (DKFZ), Heidelberg, Germany; [3] Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Goethe University, Frankfurt, Germany; [4] Department of Medicine III and Center for Healthy Aging, Technische Universität Dresden, Dresden, Germany

Iron storage diseases, such as hemochromatosis, siderosis, and bronze diabetes, result in an excess accumulation of iron in the body. While the liver serves as the primary site for iron deposition in affected individuals, cardiac complications pose the predominant threat to morbidity and mortality. Prolonged iron overload leads to progressive cardiac dysfunction, culminating in heart failure. Additionally, hemochromatosis-related iron overload induces systemic oxidative stress, endothelial dysfunction, and inflammation, collectively contributing to the onset of atherosclerosis and an elevated risk of cardiovascular events, including arrhythmias typically manifesting in advanced disease progression (Jackson et al., 2022). These arrhythmias involve precarious ventricular arrhythmias, supraventricular arrhythmias, conduction disorders, ventricular arrhythmias, and cardiac arrest, raising the susceptibility to thromboembolic incidents and sudden cardiac death (Udani et al., 2021). Although iron deposition has been proposed as a factor disrupting electrical signals and causing abnormal heart rhythms, it may not comprehensively elucidate the complex electrical phenotype. Here, we demonstrate that elevated iron levels enhance rhythmic heterogeneity in human induced pluripotent stem cell-derived cardiomyocytes and cardiac pacemaker cells, representing a common pro-arrhythmic mechanism. To further elucidate these arrhythmic effects, we conducted an analysis of iron-altered protein expression in human induced pluripotent stem cell-derived cardiac cells via MS-MS and Western blot analyses. Finally, we show altered protein cleavage of key determinants of rhythmic activity). Our findings reveal that iron overload induces remodelling of the proteome with impact on cardiac cell rythmicity.

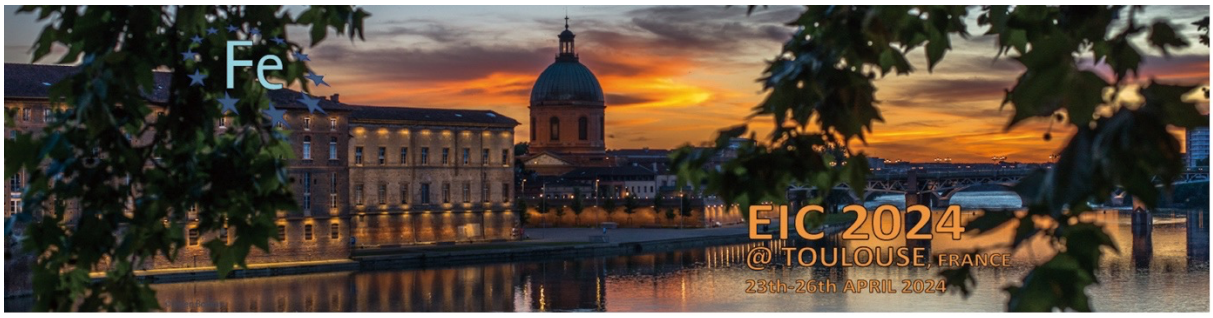


P-56 - FK506 BYPASSES THE EFFECT OF ERYTHROFERRONE IN CANCER CACHEXIA SKELETAL MUSCLE ATROPHY

MINA Erica [1], WYART Elisabeth [1], SARTORI Roberta [2], ANGELINO Elia [1], ZAGGIA Ivan [1], ANDREA Graziani [1], SANDRI Marco [2], LÉON Kautz [3], SILVESTRI Laura [4], PORPORATO Paolo [1]

[1] Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center "Guido Tarone", University of Torino, Turin, Italy; [2] Department of Biomedical Sciences, University of Padova, Padova, Italy; [3] IRSD, Université de Toulouse, INSERM, INRAE, ENVT, Univ Toulouse III - Paul Sabatier (UPS), Toulouse, France; [4] Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan, Italy

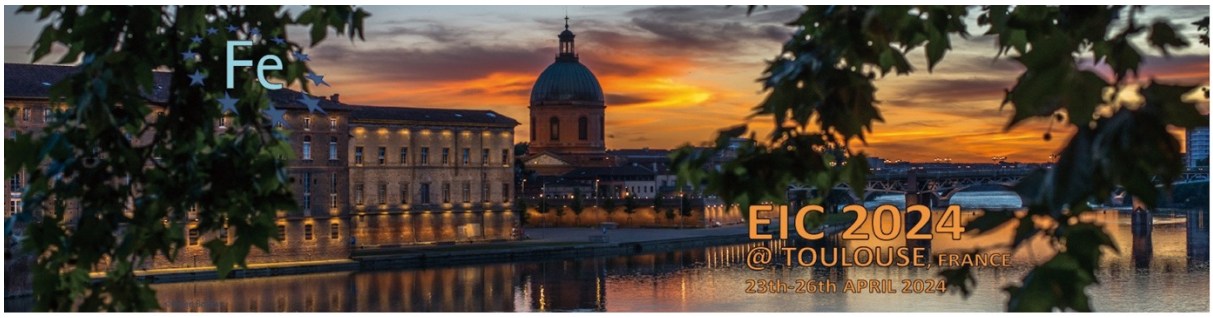
Skeletal muscle atrophy is a hallmark of cachexia, a wasting condition typical of chronic pathologies, that still represents an unmet medical need.BMP-Smad1/5/8 signaling alterations are emerging drivers of muscle catabolism, hence, characterizing these perturbations is pivotal to develop therapeutical approaches. We identified two promoters of BMP-resistance in cancer cachexia, specifically the BMP-scavenger erythroferrone (ERFE) and the intracellular inhibitor FKBP12. ERFE is upregulated in cachectic cancer patients' muscle biopsies, and in murine cachexia models, where its expression is driven by STAT3. Moreover, the knock-down of Erfe or Fkbp12 reduces muscle wasting in cachectic mice. To bypass the BMP-resistance mediated by ERFE and release the brake on the signaling, we targeted FKBP12 with low-dose FK506. FK506 restores BMP-Smad1/5/8 signaling rescuing myotube atrophy by inducing protein synthesis. In cachectic tumor-bearing mice, FK506 prevents muscle and body weight loss and protects from neuromuscular junction alteration, suggesting therapeutical potential for targeting the ERFE-FKBP12 axis.



P-57 - MYELODYSPLASTIC SYNDROMES BENEFIT FROM IRON-RESTRICTED BONE MARROW TRANSPLANT IN PRECLINICAL MODEL

ANTYPIUK Ada [1], VANCE S.zebulon [1], MARTINEZ Alberto [2], SCHAEPER Ute [2], PLATZBECKER Uwe [3], **VINCHI Francesca [1]**
[1] New York Blood Center, New York , United States; [2] Silence Therapeutics, London, United Kingdom; [3] Medical Clinic and Pliclinic 1, Hematology and Cellular Therapy, University Hospital Leipzig, Leipzig, Germany

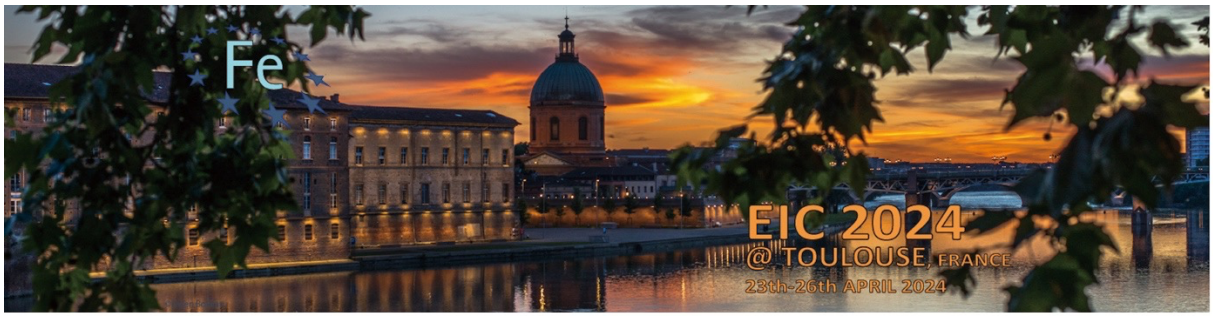
Hematopoietic stem cell transplantation remains the only fully curative therapy for myelodysplastic syndromes (MDS). Tissue iron overload and non-transferrin bound iron (NTBI) have emerged as adverse prognostic factors for post-transplant survival of MDS patients.
Recently, we showed that preparatory chemotherapy conditioning in wild-type mice resulted in peri-transplant elevation of serum iron, transferrin (Tf) saturation and NTBI formation. Pre-conditioning TMPRSS6 silencing through a GalNAc-conjugated siRNA, by increasing hepcidin levels, prevented these changes in iron parameters. Here we asked whether these observations hold true in a pathologic condition which may require HSCT, such as MDS and how iron restriction impacts on bone marrow transplant outcome. To this aim, we administered pre-conditioning the GalNAc TMPRSS6 siRNA as iron restriction strategy to CD45.2+ MDS mice, which afterwards received chemotherapy and transplant of CD45.1+ wild-type BM cells.
Importantly, conditioning exacerbated the elevated systemic iron levels in MDS mice, which was prevented by pre-conditioning TMPRSS6 silencing. Iron-restricted MDS mice receiving BMT showed a superior blood chimerism, as indicated by higher circulating CD45.1+donor cells. This resulted from a superior multilineage engraftment of hematopoietic cells in the BM and spleen of iron-restricted recipient MDS mice compared to controls. Finally, donor chimerism of HSPCs was higher in iron-restricted versus control MDS mice undergoing transplant.
Overall, these data suggest that conditioning-elicited NTBI is a critical mediator of peri-transplant toxicity and adversely impacts transplant outcome. Peri-transplant application of novel iron restriction strategies aimed at reducing conditioning-elicited NTBI, by positively affecting transplant outcome, is of potential benefit for MDS patients undergoing BMT.



P-58 - PHARMACOKINETICS AND PHARMACODYNAMICS OF AN ANTI-TMPRSS6 MONOCLONAL ANTIBODY IN MICE.

GIANNINI Silvia [1], MACDONALD Brian [1], WU Min [1]
[1] Disc Medicine, Watertown, United States

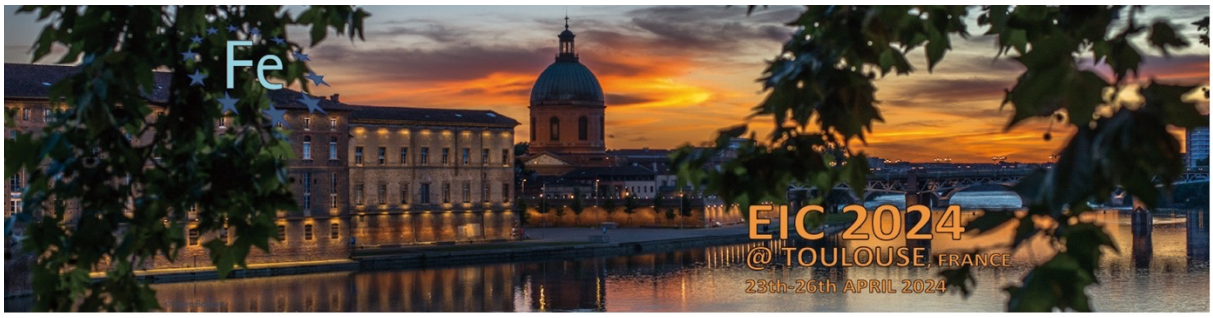
Hepcidin is considered the master regulator of iron homeostasis. Hepcidin controls iron flows by ubiquitination, internalization, and degradation of ferroportin, the sole iron exporter. The expression of hepcidin is downregulated by the transmembrane serine protease 6 (TMPRSS6) through the Bone Morphogenic protein/Suppressor of Mothers against Decapentaplegic (BMP/SMAD) signaling pathway. DISC-3405 is a novel humanized monoclonal antibody targeting TMPRSS6. The primary mode of action of DISC-3405 is to target and block the biological action of TMPRSS6 with subsequent upregulation of hepcidin leading to decreased iron absorption by enterocytes and decreased iron release from stores. A murine analog of DISC-3405, r4K12B, was generated to avoid immunogenicity in mouse studies. In this study, r4K12B was intra-peritoneally administered into wild-type mice once at four dose levels (1, 2, 5, 10 mg/kg) and PK/PD parameters were measured at day 1, 2, 3, 5, 7 and 10 post-dosing. r4K12B showed a greater than proportional PK profile. Serum hepcidin and liver HAMP mRNA levels significantly increased at day 1 post dosing and the duration of effect over time was dose-dependent. The upregulation of hepcidin was accompanied by a decrease in serum iron and transferrin saturation (TSAT), which returned to basal levels in a dose-dependent manner.
These results show that r4K12B can be used in efficacy studies in disease mouse models and further support the therapeutic potential of DISC-3405 as an iron restricting agent.



P-59 - DEFINING IRON DEFICIENCY IN RCTS: META-ANALYSIS OF THE TREATMENT EFFECT OF IV IRON

LIM Jayne [1], AL-SHAREA Annas [1], **MACLEAN Beth [1]**, ROMAN Marius [3], RICHARDS Toby [1,2]
[1] The University of Western Australia, Perth, Australia; [2] University College London, London, United Kingdom; [3] University of Leicester, Leicester, United Kingdom

Introduction: Iron deficiency (ID) is a common cause of anaemia. However, there is variability in the definition of ID. We explore the effect of IV iron based on the different definitions of ID. Methods: We updated the Cochrane review that evaluated iron therapy in patients with anaemia from inception to July 2020. The primary outcome was a change in haemoglobin (Hb). Iron status were defined as: Absolute ID (AID): Ferritin < 30 ng/ml, Functional ID (FID): Ferritin > 30ng/ml or <300 ng/ml or TSAT <20% or Anaemia: no iron studies. Results: 56 RCTs (8104 participants) were included in the meta-analysis. 26 compared IV to oral iron. 30 studies investigated IV iron. 2 studies were AID, 31 studies were FID and 23 were Anaemia. IV iron was associated with increased change in Hb (MD 0.48 (95% CI 0.06 to 0.9) and an increase in final Hb (MD: 0.85 g/dL; 95% CI, 0.34 to 1.37 g/dL; I2 = 84%) compared to oral iron. IV iron was associated with increased change in Hb (MD: 3.17 (95% CI -2.02 to 8.37) and increased in final Hb (MD: 1.21 g/dL; 95% CI, 0.15 to 2.28 g/dL; I2 =97%) and compared to placebo or no iron. Subgroup analysis by iron status did not delineate the heterogeneity. Conclusions: There is variation in the definitions of ID used for inclusion. Further research into the dynamic nature of iron metabolism in different clinical populations would allow for more reliable interpretation of trial results.



P-60 - THE USE OF DEFERIPRONE TO ALLEVIATE IRON LOADING IN MURINE ALVEOLAR-LIKE MACROPHAGES

JORDAN Ruaraidhri [1], CLOONAN Suzanne M. [1,2]

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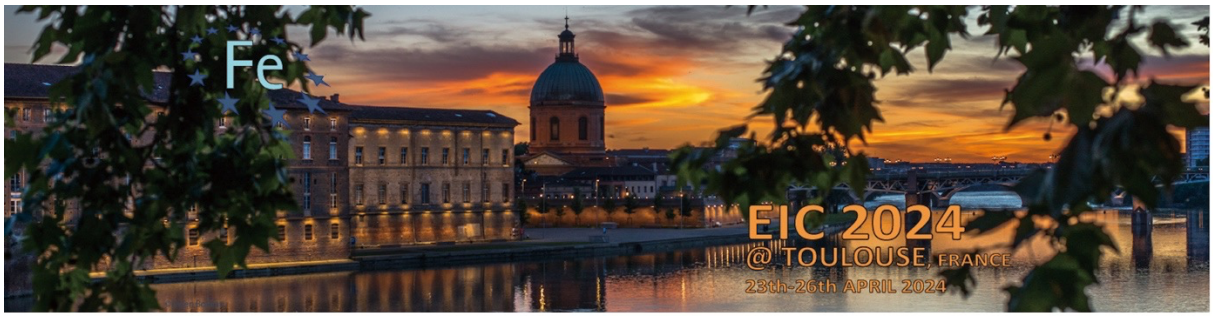
Alveolar macrophage (AM) iron loading in COPD is associated with worsened pathogen clearance and propagation of the pro-inflammatory phenotype. We have recently shown that the iron chelator deferiprone lowers iron levels in primary human AMs and may improve AM immune function in COPD patients, supporting further exploratory avenues to optimise the pharmacology of using deferiprone as a treatment for enhancing macrophage function in COPD.

This study investigated whether iron loading from different iron sources or experimental COPD could be alleviated using deferiprone in murine AMs. The maximum tolerated dose was also assessed. Foetal liver alveolar-like macrophages (FLAMs) were treated with ferric-ammonium citrate (30µg/ml), ferrous lactate (10mM), or cigarette smoke extract (10% or 15%) for 16 hours. Treatments were removed and cells were exposed to deferiprone (400µM) for 4 hours. Total cell iron (TCI) levels were assessed by graphite furnace atomic absorption spectroscopy, iron-related protein expression was assessed by immunoblotting, viability was assessed by alamarBlue assay. Human AMs were treated with deferiprone for 4 or 20 hours and viability was assessed by PrestoBlue assay. TCI was not decreased in iron-loaded FLAMs treated with deferiprone. Expression of NCOA4 and FTH1 were restored to baseline levels with deferiprone treatment. At the concentrations used in this study, deferiprone did not adversely affect cell viability. Deferiprone does not appear to decrease iron loading in this model but normalises NCOA4 and FTH1 expression, further highlighting the need for a multifaceted approach to understanding iron metabolism in COPD.

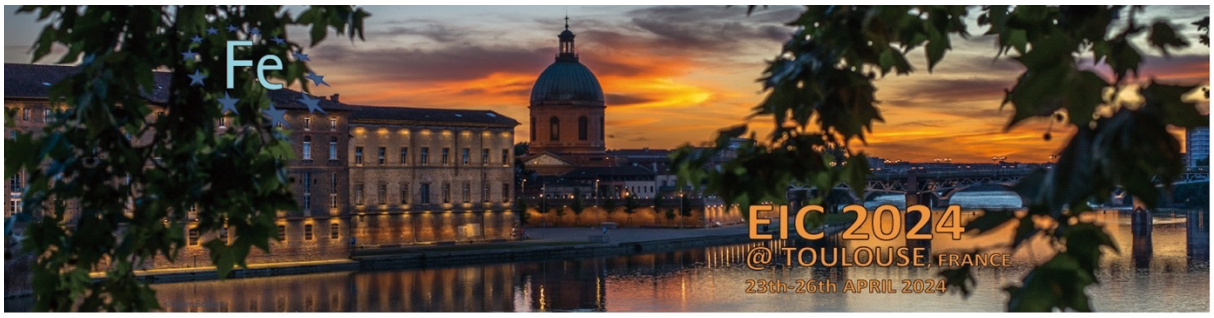


P-61 - THE FERROPORTIN INHIBITOR CPD-348 AMELIORATES THALASSEMIA SYMPTOMS IN HBB(TH3/+) MICE

ALT Carsten [1], XU Qing [1], LI Xiao-Jun [1], TRENKNER Elizabeth [1], DEGUZMAN Francis [1], LEVINE Charles [1], CHEN Yu-Wei [1], LIAOZOU Hilary [1], IKEKHUA Sarah [1], ZHANG Si Yi [1], CHANG Xiao [1], ZHANG Chenghong [1], LI Zhe [1], OKSENBERG Donna [1], CATHERS Brian E. [1]

[1] Pfizer, South San Francisco, United States

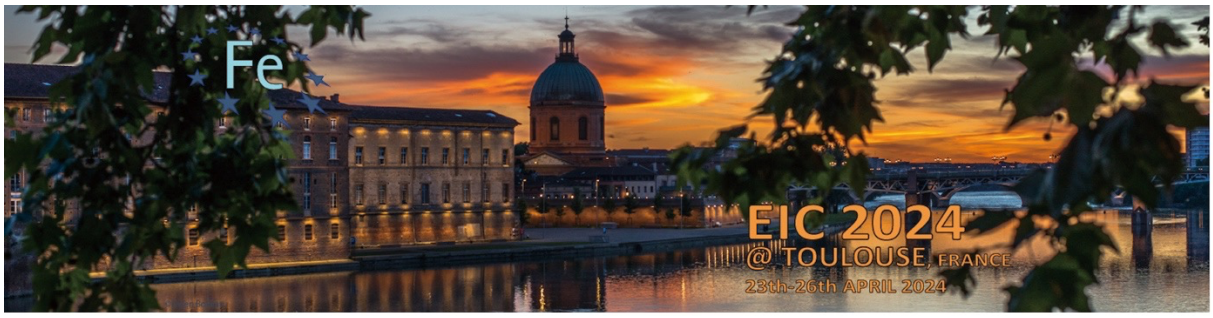
Hepcidin mimetics have been developed to improve anemia by mitigating ineffective erythropoiesis, reducing iron overload by limiting iron absorption and diminishing transfusion burden/iron overload. Previous studies have shown that hepcidin mimetics ameliorated disease symptoms in the $Hbb^{th3/+}$ model of β-thalassemia by increasing hemoglobin levels and reducing reticulocyte counts, whereas iron chelators alone did not have the same effects. In this present study we tested the novel oral small molecule hepcidin mimetic Compound 348 (Cpd-348) in comparison to vamifeport, and in presence or absence of the iron chelator deferasirox in thalassemia mice. Mice were dosed once daily for 7 weeks with Cpd-348 (2, 8, 30, or 120 mg/kg PO), vamifeport (30, or 120 mg/kg PO), or vehicle control. In a subsequent study, thalassemia mice were dosed with Cpd-348 (30 mg/kg PO) or vehicle, in presence or absence of deferasirox. Our results demonstrated that Cpd-348 alone or in presence of deferasirox ameliorated β-thalassemia symptoms in mice: Cpd-348 treatment at lower dose than vamifeport increased hemoglobin >1 g/dL, reduced reticulocytes, improved red blood cell (RBC) half-life, increased mature $CD71^-$ cells, reduced spleen weight (possibly by reducing extramedullary hematopoiesis), and improved RBC health by reducing ROS and mitochondrial content. In contrast, deferasirox alone did not have the benefits described for Cpd-348, although, as expected, effectively reduced liver iron content. In summary, Cpd-348 treatment ameliorated disease symptoms in β-thalassemia mice in absence or presence of the iron chelator deferasirox. Cpd-348 may be beneficial for the treatment of β-thalassemia and other hepcidin-related disorders.



P-62 - FERROPTOSIS IN THE HEART TISSUE OF PATIENTS WITH ATRIAL FIBRILLATION

BALUSIKOVA Kamila [1], DOSTALIKOVA Marketa [1], KOVAR Jan [1], BUDERA Petr [2], OSMANCIK Pavel [2]
[1] Centre for Research on Diabetes, Metabolism and Nutrition, Third Faculty of Medicine, Charles University, Prague, Czechia; [2] Cardiocenter, Third Faculty of Medicine, Charles University Prague and University Hospital Kralovske Vinohrady, Prague, Czechia

Atrial fibrillation is the most frequent cardiac arrhythmia with increasing prevalence and incidence. It increases the risk of heart failure, stroke, and peripheral embolism. The type of atrial fibrillation depends on how often atrial fibrillation occurs and how it responds to treatment. Paroxysmal atrial fibrillation is a brief event that usually stops in less than 24 hours but may last up to a week. Often, especially without treatment, atrial fibrillation may progress from paroxysmal to persistent to long-term. At this point, atrial fibrillation does not get better and becomes permanent. Since the heart tissue of the patients undergoes long-term stress conditions, some type of programmed cell death was expected to be detected in the heart samples.
Our study included 13 patients with permanent atrial fibrillation, 10 patients with paroxysmal atrial fibrillation, and 11 corresponding controls. The left atrial appendage of each patient was resected during the surgical ablation. Tissue samples were processed for total protein isolation and protein levels were analyzed using western blot analysis.
In our analyses, we have tested protein levels of iron metabolism-related molecules, molecules to be known for their role in ferroptosis as well as apoptotic cell markers. However, we have detected only slight changes that were not statistically significant and therefore have not obtained significant indication of active ferroptosis in our samples.
This study was funded by a research program of Charles University in Prague COOPERATIO 31 and by Programme EXCELES, ID Project No. LX22NPO5104 - Funded by the European Union (Next Generation EU).

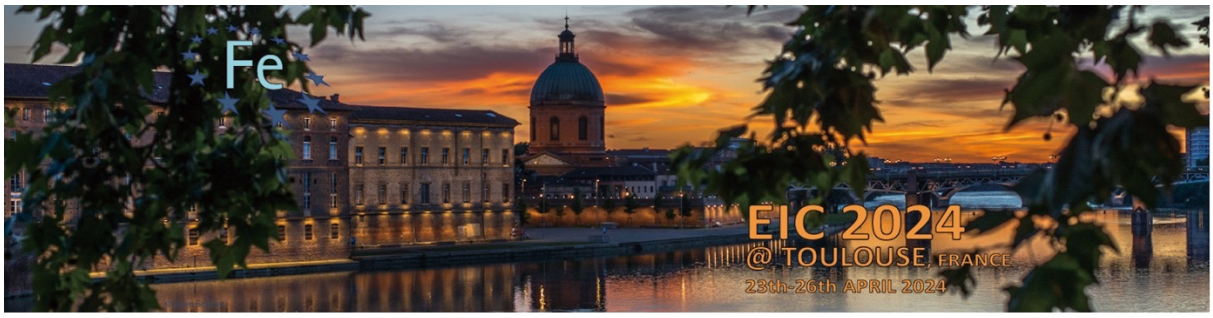


P-64 - Deciphering ferroptosis in mucopolysaccharidosis III (MPSIIIB) mouse model

LARRIBAU Mathilde [1], ROUAHI Myriam [1], SANTIAGO Christophe [1], AUSSEIL Jérôme [1], KARIM Zoubida [1]
[1] Institute for Infectious and Inflammatory Diseases (Infinity)- INSERM U1291, 31024 Toulouse, France

Sanfilippo syndrome (MPSIII) is caused by lysosomal dysfunction and accumulation of abnormal heparan sulphate in brain, leading to neurodegeneration. Ferroptosis is an iron-dependent cell death driven by the production of reactive oxygen species and lipids peroxidation. Ferroptosis is associated with various neurodegenerative diseases, where iron accumulates due to intrinsic brain dysregulation of iron metabolism. We have previously shown that, iron dysregulation in the cerebral cortex is also a distinctive feature of MPSIII pathogenesis, but it remains to be determined whether MPSIII neurons are affected by ferroptosis. Thus, we investigate the ferroptotic signature in groups of WT and MPSIIIB mice at 5 months of age. The results showed several specific markers of ferroptosis. The expression level of TFR1, an iron import was decreased, brain iron content was increased but ferritin levels was increased, confirming iron accumulation in MPSIIIB brain. The xc-/GPX4 negative ferroptosis regulatory system was reduced. Oxidative homeostasis was affected as shown by increased expression of SOD2, SIRT3, iNOS, and nNOS. Finally, the expression of lipid peroxidation genes (ACSLl4, LPCAT3) was increased, indicating accumulation of peroxide lipids.
Furthermore, we observed a significant increase in the abundance of the iron exporter, ferroportin in MPSIIIB brain, but immunohistological labeling indicated an increase in this protein only in neurons (identified by NeuN markers). However, FPN exhibited an abnormal intracellular-vacuolar location, suggesting inadequate targeting to the plasma membrane and implicating its role in neuron iron accumulation.

In summary, we conclude that in MPSIIIB, neuronal alterations are directly associated with iron accumulation and ferroptosis.

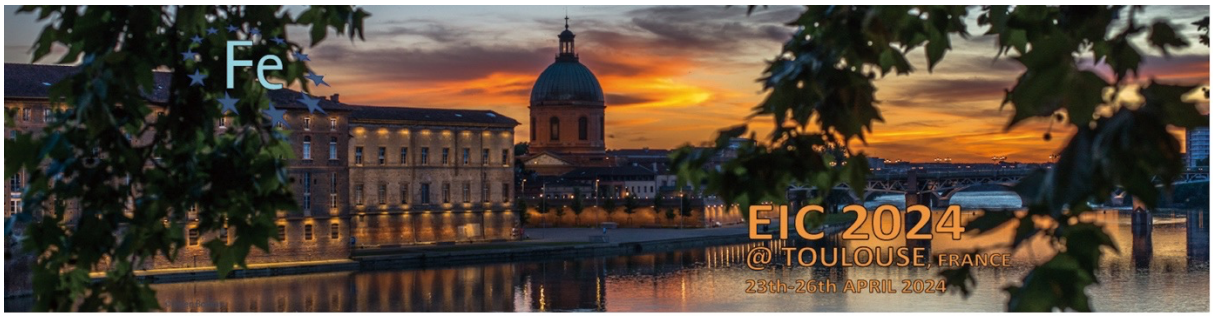


P-66 - ATR inhibitor camonsertib (RP-3500) suppresses early-stage erythroblasts by mediating ferroptosis

LEVY Maayan [1], FERRARO Gino [2], LI Li [2], HAN Yongshuai [3], FEDERICO Giorgia [5], VARRICCHIO Lilian [1], FOURNIER Sara [2], AN Xiuli [3], HOFFMAN Ronald [1], FRETLAND Adrian [4], CARLOMAGNO Francesca [5], ROULSTON Anne [4], **GINZBURG Yelena [1]**

[1] Icahn School Of Medicine At Mount Sinai, New York, United States; [2] Repare Therapeutics Inc, Saint-Laurent, Quebec, Canada; [3] New York Blood Center, New York, United States; [4] Repare Therapeutics Inc, Cambridge, Massachusetts, United States; [5] University of Naples, Naples, Italy

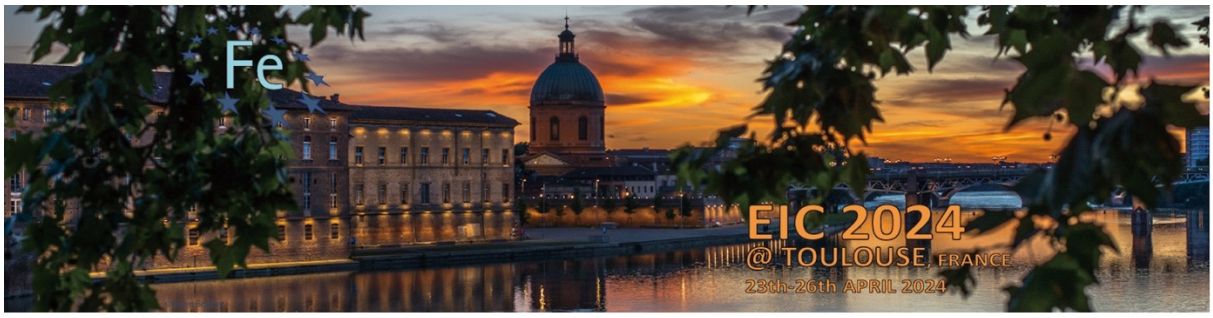
Ataxia telangiectasia mutated and Rad3-related kinase (ATR) mediates cellular response to replication stress and DNA damage. Camonsertib is a potent and selective ATR inhibitor (ATRi) with strong pre-clinical efficacy and promising clinical activity (NCT04497116) in cancer patients but leads to severe anemia. High iron requirements are essential for heme synthesis during erythropoiesis, specifically increasing erythroblast vulnerability to iron-mediated oxidative damage. We evaluate intracellular iron homeostasis by measuring effects of camonsertib on iron uptake (TFR1), storage (ferritin), and transport (PCBP1/2 and NCOA4). Iron accumulation in ferritin and NCOA4-mediated ferritinophagy is essential for normal erythropoiesis while unmitigated iron release from ferritin increases the sensitivity of cells to ferroptosis, a non-apoptotic iron-dependent form of cell death resulting from iron-mediated lipid peroxidation. We hypothesize that camonsertib induces ferroptosis in erythroblasts, causing anemia. We demonstrate that erythroblast depletion occurs following camonsertib in mice and is reversible with erythroblast regeneration during dose interruption. In addition, using human CD34+ cells induced to differentiate to erythroblasts in vitro, cells treated with camonsertib demonstrate a dose-dependent decrease in proliferation and differentiation and increase in apoptosis and reactive oxygen species (ROS). Specifically, decreased TFRC and PCBP1/2 as well as increased NCOA4 and FTH expression is consistent with enhanced ferritinophagy. In addition, we demonstrate a dose-response increase in lipid peroxidation and 4HNE in camonsertib-treated erythroblasts. Finally, consistent with increased ferroptosis, GPX4 expression decreased and the addition of ferristatin-1 prevents increased ROS in camonsertib-treated erythroblasts. Taken together, we demonstrate ferroptosis induction in camonsertib-treated erythroblasts as a potential underlying cause of ATRi-induced anemia.



P-67 - Development of peptide-based tools to measure cellular free heme after UVA irradiation

GAO Nan [1], PASCU Sofia [2,3,4], POURZAND Charareh [1,2,3], EGGLESTON Ian [1]
[1] Department of Life Sciences, University of Bath, Bath, United Kingdom; [2] Centre for Therapeutic Innovation, University of Bath , Bath, United Kingdom; [3] Centre for Bioengineering and Biomedical Technologies, University of Bath, Bath, United Kingdom; [4] Department of Chemistry, University of Bath, Bath, United Kingdom

Heme plays a vital role in cell biology and the dysregulation of heme levels is implicated in a wide range of diseases. Currently however there is a lack of convenient chemical tools that can be used to measure the changes in heme levels in biological media or in live cells. Exposure of skin cells to solar UVA radiation has been shown to result in elevated levels of free heme and upregulation of Heme oxygenase-1 (HO-1), the oxidant-inducible enzyme that catalyses the breakdown of pro-oxidant heme. There is therefore considerable interest in developing new probes to study such UVA-induced changes in heme levels and gain a greater understanding of the detrimental and potentially beneficial effects of UVA on human skin. The aim of this work is to explore the development of probe molecules that are derived from natural heme-binding proteins. We will describe a tryptophan-containing peptide probe based on a peptide sequence from the natural heme-binding protein, Bach-1 a transcription factor that acts as a negative regulator of the HO-1 gene. The fluorescence of the tryptophan residue can be quenched upon binding of heme to the probe peptide providing a direct readout of heme levels. Here, we describe an efficient and scalable synthesis of the novel fluorescent tryptophan analogue, 7-aza-tryptophan that is incorporated into the Bach-I derived peptide by solid phase peptide synthesis. This peptide probe can sensitively measure changes in heme levels in human dermal fibroblasts, FEK4 cells following exposure to increasing levels of UVA irradiation (up to 500 kJ/m^2).



P-68 - A HEME-DEPENDENT MITOCHONDRIAL FAILURE CONTRIBUTES TO THE PATHOGENESIS OF FLVCR1-RELATED DISEASES.

BERTINO Francesca [1], GRASSO Eleonora [1], KOPECKA Joanna [2], **ZANIN VENTURINI Diletta Isabella [1]**, BASNET Ram [3], BELLINI Stefania [4], MIGNANI Luca [3], ZHAO Boxun [5], HENTSCHEL Andreas [6], MAGNANI Francesca [7], ABALAI Raluca Elena [1], MCCOURT Emily [8], KOBEL Heike [9], LARSON Austin [10], ROOS Andreas [11], W. YU Timothy [5], FINAZZI Dario [3], CHIABRANDO Deborah [1], TOLOSANO Emanuela [1]

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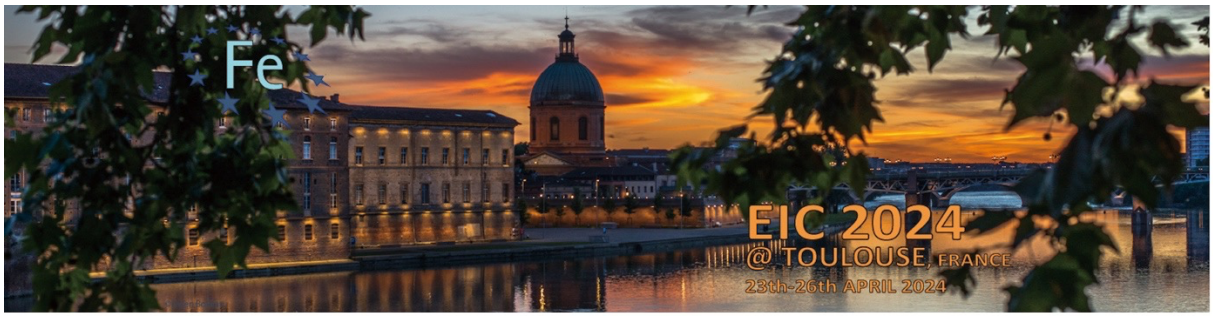
FLVCR1-related diseases encompass a group of rare genetic disorders characterized by sensory neuropathy, sensory ataxia and retinitis pigmentosa, that are caused by mutations in the Feline Leukemia Virus Subgroup C Receptor 1 (FLVCR1) gene. Evidence suggest that mitochondrial failure represents a key pathogenetic mechanism underlying FLVCR1-related diseases and that patients may benefit from therapeutic strategies boosting mitochondrial energy metabolism. However, a comprehensive understanding of the cause responsible for mitochondrial dysfunction is mandatory to choose the best therapeutic approach in future. Considering that FLVCR1 mutations impact on both heme and choline metabolism, we sought to understand whether the mitochondrial failure in patients' cells can be attributed to the alteration of one of these two processes and whether pharmacological treatments are effective in restoring mitochondrial function. Our findings reveal mitochondrial dysfunction and heightened lipid peroxidation in patient-derived fibroblasts and zebrafish morphants. Rescue experiments demonstrate that targeting heme metabolism fully reinstates TCA cycle flux, oxidative phosphorylation, and mitochondrial ATP content in patient fibroblasts, while choline supplementation only partially restores mitochondrial ATP content. Intriguingly, both interventions significantly mitigate lipid peroxidation in patient fibroblasts. Although the potential role of choline cannot be discounted, our results strongly suggest that a heme-dependent mitochondrial failure is a pivotal factor in the disease pathogenesis. This study defines FLVCR1-associated sensory neuropathy as a mitochondrial disorder specific to certain tissues, representing a significant stride towards identifying therapeutic approaches for this rare disease.

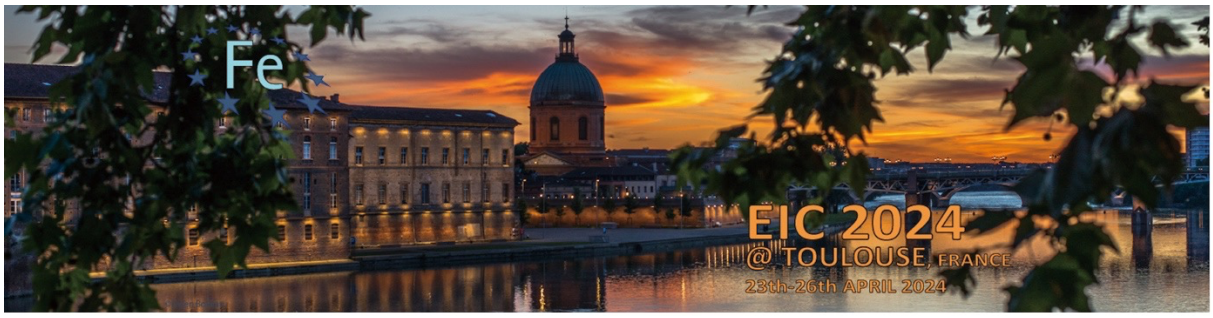


P-69 - UNRAVELING IRON METABOLISM IN CANCER: INSIGHTS INTO FLVCR1A AND HEME BIOSYNTHESIS

FIORITO Veronica [1,5], ALLOCCO Anna Lucia [1,5], RIGANTI Chiara [2], FERRAUTO Giuseppe [3], BELLINI Stefania [4], FORNI Marco [1], AMMIRATA Giorgia [1], AMBROGIO Chiara [1], TOLOSANO Emanuela [1]

[1] Molecular Biotechnology Center "Guido Tarone", Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy; [2] Department of Oncology, University of Torino, Torino, Italy; [3] Molecular Imaging Center, Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy; [4] Laboratory of Diabetic Nephropathy, Department of Medical Sciences, University of Torino, Torino, Italy; [5] These authors contributed equally to this work, ., Italy

Dysregulated iron metabolism is a hallmark of cancer. Moreover, increased trapping of iron in protoporphyrin IX, to generate bioavailable heme-iron, is considered a tumor dependency, especially in KRAS mutated pancreatic and lung cancers. While the mechanisms orchestrating the rise in labile iron levels in proliferating cells have been extensively studied, the factors triggering heme biosynthesis activation in tumors remain poorly understood. Our research elucidates the essential role of the plasma membrane transporter Feline Leukemia Virus subgroup C Receptor 1a (FLVCR1a) in driving heme biosynthesis in proliferating cells: depletion of this transporter leads to decreased ALAS1-dependent heme biosynthesis, hindering the development of a glycolytic profile in KRAS-mutated tumor cells and consequently reducing tumor initiation, growth, and dissemination. We propose that enhanced expression of FLVCR1a in tumor cells is part of the rewiring of iron metabolism and serves as one of the triggering events harnessed by cells to transition from a non-proliferative to a proliferative state. The recent revelation that this transporter, previously thought to export heme, can import choline and/or ethanolamine opens up new avenues, suggesting that oncogene-induced modulation of lipid metabolism could be the initial trigger for stimulation of heme biosynthesis, in order to initiate and sustain proliferation.



P-70 - Early diagnosis of aceruloplasminemia in infancy through NGS sequencing

CUNAT Séverine [1], GAUTHIER VASSEROT Alexandra [2], JOLY Philippe [3], GIANSILY BLAIZOT Muriel [1], **AGUILAR MARTINEZ1 Patricia [1]**

[1] Laboratory of hematological biology and expert center on rare iron disorders, Hôpital Saint Eloi, CHU of Montpellier, Montpellier, France; [2] Institut d'hématologie et d'oncologie pédiatrique, Hospices Civils de Lyon, Lyon, France; [3] Hospices Civils de Lyon, Service de biochimie et de biologie moléculaire Grand Est, Bron, France

Aceruloplasminemia is a rare autosomal recessive disorder of iron metabolism, linked to mutations in the *CP* gene. Most diagnoses are made in adulthood in the context of neurological manifestations and diabetes mellitus. The disease is usually accompanied by moderate microcytic anemia, which is not the main clinical feature.

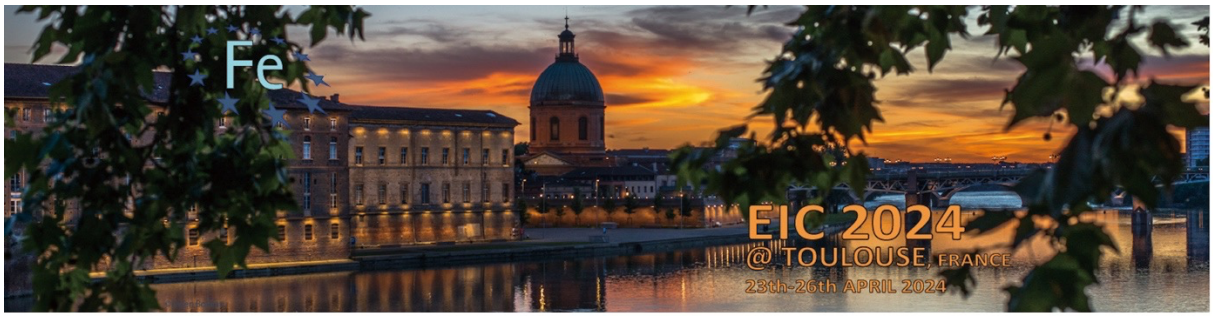
We report here the discovery of aceruloplasminemia in a 2-year-old girl.

The child was initially examined at the age of 6 months for microcytic hypochromic anemia without overt iron-deficiency. Nevertheless, oral iron administration increased blood hemoglobin from 6 to 8 g/dL, and then, stayed at this value. There was no dysmorphic syndrome and no mental retardation nor growth delay. Globin gene studies were normal. Bone marrow examination ruled out a sideroblastic anemia, but showed severe iron depletion. Serum ferritin was elevated (>100 µg/L), serum iron and transferrin saturation were very low, with no biological inflammatory syndrome.

Analysis of a NGS panel of genes involved in iron and red blood cell disorders identified a homozygous *CP* gene variant (p.(Glu587ArgfsTer37)). This variant is not referenced in literature nor in databases. However, it is located in one of the cupredoxin protein domains. It results in a frameshift from codon 587 onwards with a stop codon 37 amino acids later. This change is therefore likely to significantly alter the protein's function and ferroxidase activity.

The question now is what treatment could be given to this child, to avoid the constitution of brain iron overload which is key to the prognosis of the disease.

Acknowledgements: ERN-EuroBloodNet



P-71 - BONE DEFICITS IN RESPONSE TO IRON ADMINISTRATION BETWEEN HFE HEMOCHROMATOSIS AND WILDTYPE MICE

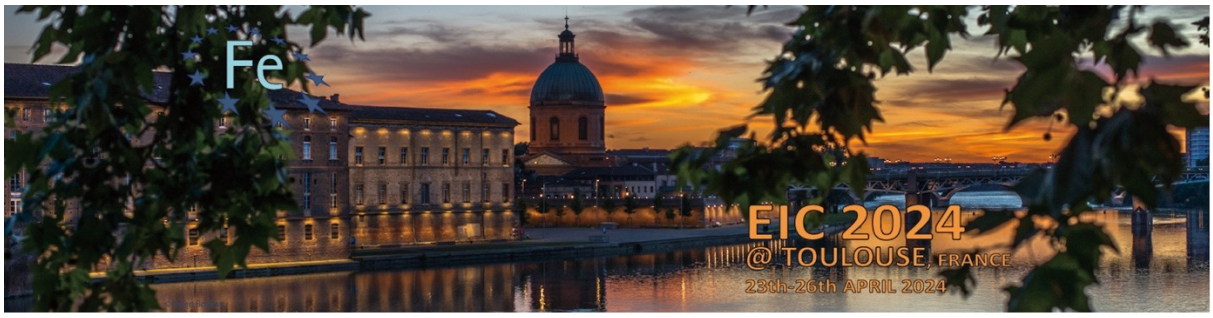
STEELE-PERKINS Peter [1], WAGNER Alessa [2], YILMAZ Dilay [1], VUJIC SPASIC Maja [1]
[1] University Ulm, Ulm, Germany; [2] Universitätsklinikum Ulm, Ulm, Germany

HFE-hemochromatosis (HH) is a hepcidin dysregulation disorder resulting in systemic iron overload. Associative studies in patients and preclinical models reported a correlation between HFE/Hfe-HH and bone dysfunction such as osteoporosis, osteopenia, and arthropathy. Interestingly, removal of iron via phlebotomy does not appear to improve bone anomalies, suggesting that additional triggers of bone loss are present. Moreover, we previously showed systemic iron overload in Hfe-HH mice is insufficient to cause bone loss.

Our study investigates if Hfe-/- mice are more susceptible to bone deficits than wildtype mice (Wt), when additional triggers of bone loss are present. Parenteral administration of iron is known to cause bone loss in Wt mice. We intraperitoneally injected Hfe-/- and Wt mice (sex/age-matched, C57Bl/6J background) with iron dextran at 1g/kg and 0.1g/kg for 8 weeks, and assessed bone status.

µCT analysis revealed a strong bone deficit in both trabecular and cortical bone of Hfe-/- and Wt male mice femurs after 8 weeks of injection with 1g/kg. Of particular interest, are however the effects of the lower iron dose (0.1g/kg). We show bone deficits in Hfe-/- mice that are not replicated in the Wt mice: Femoral and L5 vertebra BT/TV were decreased, and cortical bone was also unilaterally impacted, with thickness decreasing only in Hfe-/-. These effects were observed despite the lack of expected liver impairment such as fibrosis.

Our data implies that Hfe-/- mice are more prone to disruption of bone status than wild-type counterparts. Deciphering the underlying mechanism(s) of this discrepancy is our current goal.



P-72 - CONSENSUS INITIATIVE FOR THE DEFINITION OF PHENOTYPIC DIAGNOSTIC CRITERIA FOR HEMOCHROMATOSIS

TROPPMAIR Maria Rosina [1], PORTO Graca [2], BARDOU-JACQUET Edouard [3], SHEARMAN Jeremy [4], RYAN John D. [5], GIRELLI Domenico [6], CORRADINI Elena [7], GRIFFITHS Bill [8], VALENTI Luca [9], GINZBURG Yelena [10], BUSTI Fabiana [6], PLAIKNER Michaela [1], HENNINGER Benjamin [1], GANDON Yves [3], ZOLLER Heinz [1], TILG Herbert [1]

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Background

The finding that hemochromatosis is caused by a dysregulation of the hepcidin-ferroportin axis has promoted an update in disease nomenclature without providing practical diagnostic criteria. Iron overload in hemochromatosis is progressive in the liver, while sparing the spleen. R2* Magnetic Resonance Imaging (MRI) has replaced liver biopsy for iron quantification. R2* is directly proportional to liver iron and linearly increases with magnetic field strength.

Aims

The aim of this initiative is to establish phenotypic diagnostic criteria for hemochromatosis and develop thresholds for liver and spleen R2*/T in patients with iron overload and homozygosity for the C282Y variant to develop diagnostic criteria applicable for patients with suspected non HFE-hemochromatosis.

Methods

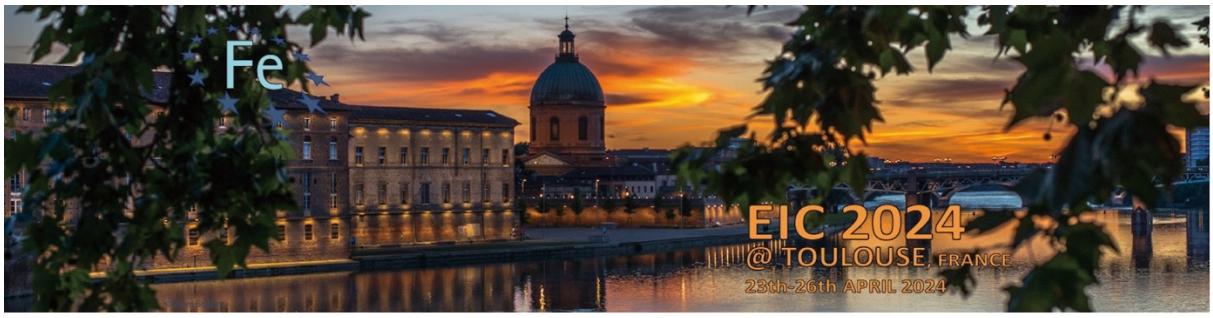
In this ongoing multi-center retrospective study, we collect liver and spleen MRI (R2*/Tesla) and serum iron parameters in patients homozygous for C282Y. In the study cohort, hemochromatosis was defined by homozygosity for C282Y and transferrin saturation (TSAT) > 50% in males and > 45% in females.

Results

In 143 C282Y homozygous patients who met the inclusion criteria, median ferritin was 738.5mg/l (460.8; 1679.0) and median TSAT 83% (69.3; 87.0). Median liver R2*/Tesla was 120.6s-1*T-1 (54.5;228.6), median spleen R2*/Tesla 24.0s-1*T-1 (17.5; 32.3).

Conclusion

We propose 2 out of 3 of the following criteria for the phenotypic hemochromatosis diagnosis: (i) C282Y homozygosity, (ii) elevated TSAT and (iii) liver R2* >100s-1T-1 with spleen R2* <50s-1T-1. If appropriately validated, these criteria could be used as thresholds for treatment indication and for referral to 2nd level genetic testing in patients with suspected non-HFE hemochromatosis.



P-73 - Haemochromatosis HFE genotypes and chondrocalcinosis: Early data from analysis in UK Biobank

BANFIELD Lucy [1], KNAPP Karen [1], PILLING Luke [2], MELZER David [2], ATKINS Janice [2]
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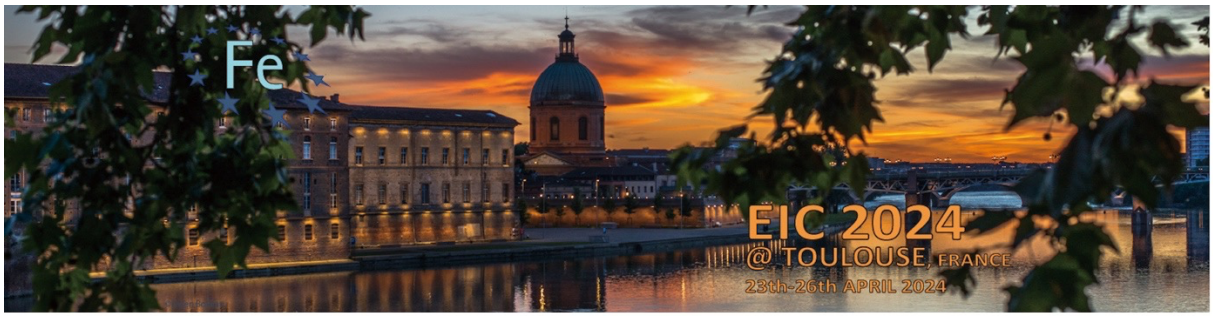
Background:
The iron-overload disorder haemochromatosis is primarily caused by the homozygous HFE p.C282Y variant. Musculoskeletal changes including arthropathy and chondrocalcinosis (cartilage calcification) are well-recognised features of the clinical disease, however, less is known about chondrocalcinosis formation in those with the p.C282Y variant. We used UK Biobank iDXA (dual-energy X-ray absorptiometer) images to assess for chondrocalcinosis in the knee and to explore any association with p.C282Y genotype.

Methods:
We used data from 144 p.C282Y homozygotes of European genetic ancestry (aged 48 to 80years) and 144 controls matched for age, sex, and BMI. Within these 288 participants, 264 had relevant knee iDXA (GELunar, Bedford) imaging. These images were reviewed for radiological evidence of chondrocalcinosis by an experienced reporting radiographer. Logistic regression models assessed associations between the p.C282Y homozygous genotype and chondrocalcinosis. Analyses were stratified by sex and adjusted for age.

Results:
Male p.C282Y homozygotes had significantly increased odds of radiological chondrocalcinosis in either imaged knee when compared with matched controls without HFE haemochromatosis mutations (OR=12.59 [95% CI:1.51-104.70] p=0.019).
Female p.C282Y homozygotes did not have increased odds of chondrocalcinosis in either knee, (OR=3.51 [95% CI:0.69-17.79] p=0.129). Larger sample sizes are needed to increase power and we plan to review further iDXA images within UK Biobank, and also to explore any associations with an arthritis diagnosis.

Conclusion: In this community genotyped sample, male p.C282Y homozygotes demonstrated significantly increased odds of developing chondrocalcinosis within the knee. These results may support further investigations such as serum ferritin levels when chondrocalcinosis is identified on imaging.



P-75 - ALBUMIN MODIFICATION IN HEMOCHROMATOSIS

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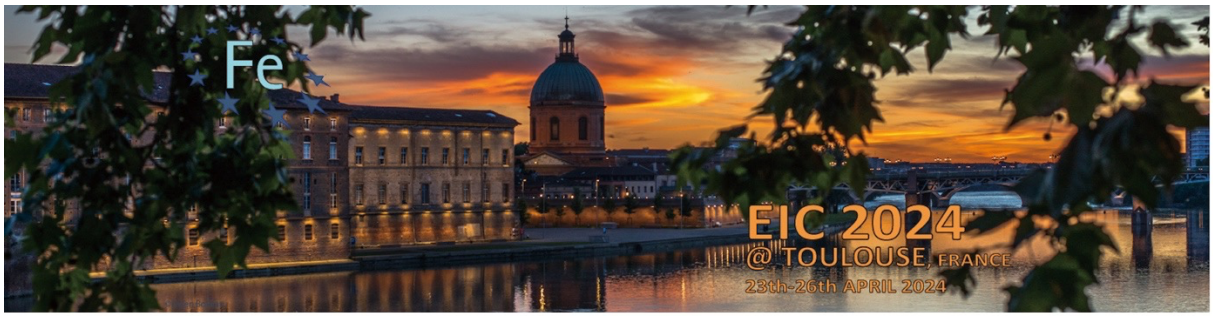
NTBI is present under the form of a heterogenous mixture of species, composed of Low Molecular Weight species (LMW-NTBI) and High Molecular Weight species (HMW-NTBI). Albumin (HSA) has been proposed as the main candidate for the formation of HMW-NTBI, with protein post-translational modifications (PTMs) playing a role in this interaction.
Here in, we aimed to study HSA PTMs by mass spectrometry, namely glycation and oxidation, to evaluate their contribution to NTBI levels in blood serum samples. Serum from individuals homozygous for the p.C282Y variant in HFE, as well as samples from healthy blood donors, were utilized to characterize HSA PTMs and study their correlation with NTBI levels.

Results show that glycation levels were altered in HH patients, which showed higher PTM site occupancy values in both K233 and K525 in comparison with controls. HSA oxidation was also altered in HH patients, namely those under intensive treatment, with significantly higher oxidation values for M87 (2.6%) in comparison with controls (1.0%). Glycation values for K525 and oxidation values for M87 and M298 showed a positive correlation with NTBI levels. These results may reveal the unspecific iron binding sites in HSA. Additionally, these results positively indicate an interaction between HSA and serum iron levels, supporting the role of HSA in NTBI speciation, in vivo.

Funding: The work was financially supported by the eQuaNTI project (2022.06328.PTDC), funded by FCT/MCTES through national funds.

Acknowledgments: FCT/MCTES through the project UIDB/50006/2020 | UIDP/50006/2020.



P-76 - TRANSFERRIN IRON-LOBE DISTRIBUTION AND POST-TRANSLATIONAL MODIFICATIONS IN HEMOCHROMATOSIS

RAMOS Eduardo [1], PORTO Graça [2,3,4], RANGEL Maria [5], SILVA André [1,5]

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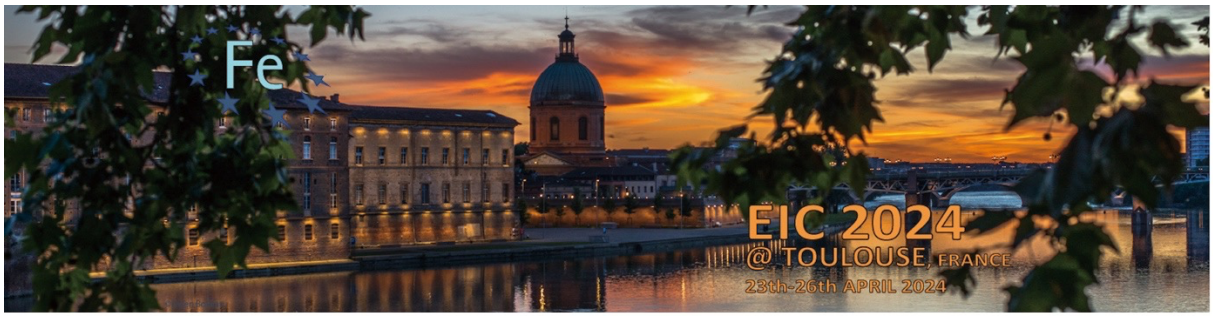
Hereditary Hemochromatosis (HH) is a widespread autosomal recessive disorder in Northern European populations. It is characterized by high iron absorption, systemic iron overload, elevated iron stores, and increased oxidative stress. The iron transport protein Transferrin (Tf) is necessary for cellular iron internalization, and systemic distribution. Post Translational Modifications (PTMS) have been identified in Tf and have the potential to modify its function and iron-binding capacity in a physiologically relevant manner.

This study focused on assessing asymmetrical iron lobe distribution in Tf and investigating potential changes in HH patients compared to healthy individuals. We also aimed at identifying PTMs in Tf binding sites and their interplay with iron lobe distribution. Serum samples from p.C282Y homozygous HH patients, undergoing phlebotomy and samples from healthy blood donors were analysed using Urea-gel electrophoresis and Mass Spectrometry-Based Proteomics to study iron lobe distribution and Tf modifications.

HH patients exhibited significantly higher Tf-Fe_2 levels (65%) and lower Apo-Tf levels (11%) compared to healthy individuals (12% Tf-Fe_2, 46% Apo-Tf). It was also found that patients undergoing intensive treatment presented significantly lower Tf-Fe_N levels (7.5%) when compared to controls (20%). Tf PTMs were identified which might impact Tf function and alter its iron-binding dynamics and lobe distribution.

These findings might help to enlighten how PTMs can potentially compromise Tf iron-binding capacity. However, a higher understanding of Tf biochemistry is needed to explain differential clinical outcomes and disease-specific patterns of iron deposition.

Funding: *The work was financially supported by the eQuaNTI project (2022.06328.PTDC), funded by FCT/MCTES through national funds*.



P-77 - CENTRAL ADIPOSITY AND HAEMOCHROMATOSIS CLINICAL PENETRANCE IN HFE P.C282Y HOMOZYGOTES IN UK BIOBANK

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[1] University of Exeter, Exeter, United Kingdom

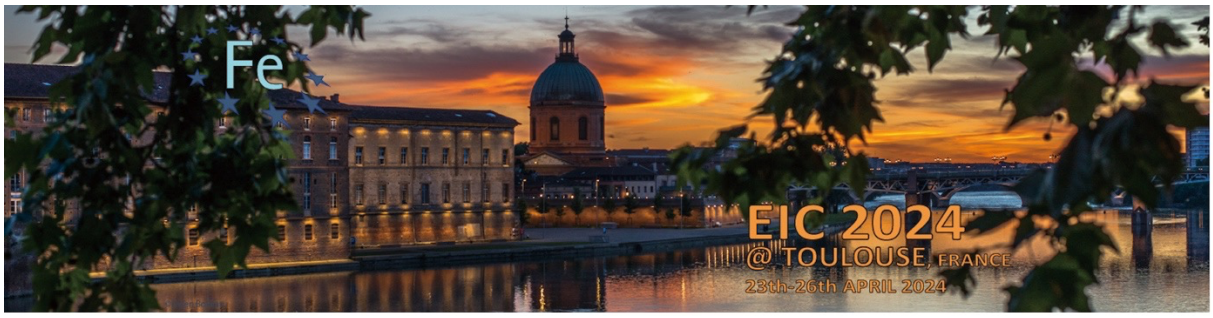
Background: Haemochromatosis, primarily caused by HFE p.C282Y homozygosity (+/+), is associated with similar clinical outcomes to obesity, including liver disease and diabetes. We assessed central adiposity's impact on clinical penetrance in p.C282Y+/+ in a large community cohort.

Methods: We studied 2,899 p.C282Y+/+ UK Biobank participants of European genetic ancestry, with a mean 13.3-year follow-up. Cox models assessed the association between high waist-to-hip ratio (WHR; >=0.96 for men, >=0.85 for women) and risk of incident outcomes, stratified by sex and adjusted for age and genetic components. Cumulative incidence of outcomes was estimated to age 80 years.

Results: Male p.C282Y+/+ with high WHR had greater liver fibrosis/cirrhosis risk compared to normal WHR (HR=4.13, 95%CI: 2.04-8.39, p=8.4*10-5); cumulative incidence to age 80 was 15.00% [95%CI: 9.81-22.57] vs 3.85% [95%CI: 1.93-7.60] in normal WHR, and also liver cancer (HR=2.57, 95%CI: 1.24-5.33, p=1.10*10-2; 9.15% [95%CI: 5.68-14.57] vs 3.57% [95%CI: 1.92-6.58]), non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes. Female p.C282Y+/+ with high WHR had greater risk and cumulative incidences of liver fibrosis/cirrhosis (HR=9.17, 95%CI: 2.51-33.50, p=3.8x10-7; 4.59% [95%CI: 2.54-8.24] vs 0.58% [95%CI:0.19-1.80]), NAFLD (HR=5.17, 95%CI: 2.48-10.78, p=1.2x10-5), and joint replacement (HR 1.42, 95%CI: 1.03-1.96, p=0.03).

In a model analysing the whole cohort (all HFE genotype groups, n=451,270), there was a significant interaction between having a high WHR and p.C282Y+/+ genotype for risk of all statistically significant outcomes.

Conclusion: In p.C282Y+/+ males and females, high WHR strongly predicts clinical penetrance. Interventions to reduce central adiposity should be tested in p.C282Y+/+ to improve associated clinical outcomes.



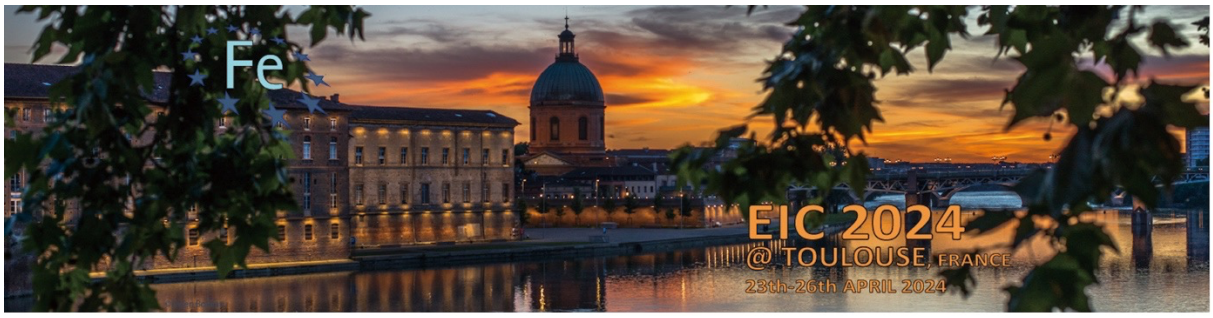
P-78 - THE ROLE OF THE HEMOCHROMATOSIS PROTEIN HFE IN MACROPHAGE METABOLISM

KNOOP Paul [1], AKDOGAN Banu [2], ZISCHKA Hans [2,3], VUJIC SPASIC Maja [1]
[1] University Ulm, Ulm, Germany; [2] Helmholtz Munich, Munich, Germany; [3] Technical University Munich, Munich, Germany

Mutations in the iron-metabolic gene HFE are the most common cause for hereditary hemochromatosis (HH). The disease is characterized by insufficient production of the iron hormone hepcidin, leading to harmful, increased iron deposition in multiple tissues. Non-liver resident macrophages lacking Hfe show low intracellular iron independently of hepatic hepcidin expression1. Mice with a constitutive (Hfe-/-) or selective myeloid-Hfe deficiency (HfeLysMCre) show an improved survival during endotoxin shock and Salmonella infection1,2, linking Hfe in macrophages to inflammatory responses. Since iron content and metabolic reprogramming are hallmarks of macrophage phenotype and function, we postulate that Hfe acts in a macrophage specific manner and influences metabolism and mitochondrial function.
To characterize the metabolic phenotype of macrophages lacking Hfe, we performed a real-time respirometry analysis (Seahorse, Agilent) on bone marrow derived macrophages from HfeLysMCre and Hfe-/- mice. Enzymatic activity and metabolite abundance measurements revealed further insights. Hfe-deficient macrophages show impaired glycolysis and decreased respirational function. Activity of aconitase and succinate-dehydrogenase (SDHB) are compromised, leading to increased succinate levels. Macrophages lacking Hfe, show altered mitochondrial and metabolic capacities leading to a bioenergetic shift and along with the iron-poor phenotype, could explain the differential immune response and possibly revealing a macrophage-specific action of Hfe.

1. Tangudu, N. K. et al. Macrophage-HFE controls iron metabolism and immune responses in aged mice. Haematologica (2021).

2. Nairz, M. et al. Cell-specific expression of Hfe determines the outcome of Salmonella enterica serovar Typhimurium infection in mice. Haematologica (2020)



P-79 - CLINICAL AND GENETIC CHARACTERISTICS OF A PROSPECTIVENONHFE C282Y HEMOCHROMATOSIS COHORT

HAMDI-ROZE Houda [1], DETIVAUD Lenaick [1], GANDON Yves [1], LOREAL Olivier [1], ROPERT-BOUCHET Martine [1], **BARDOU-JACQUET Edouard [1]**

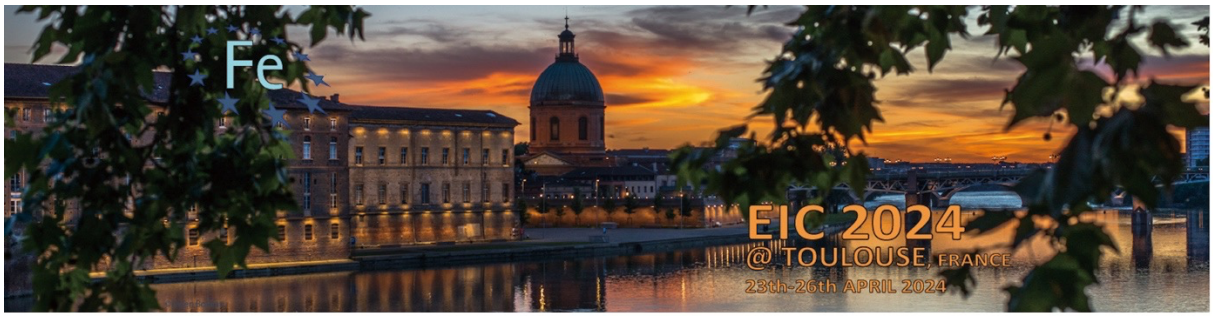
[1] CHU Rennes, Rennes Cedex 9, France

Background:
Rare hemochromatosis are mainly described using retrospective study and cases reports. A new consensus classification allows diagnosis encompassing broader definition. We aimed to describe a nationwide prospective cohort of patients with non HFE C282Y hemochromatosis.

Method:
Patients with Saturation>50% and Liver iron content >100µmol/g, without secondary causes of iron overload were prospectively included in ten centers. Liver iron content (LIC) MRI interpretation, biochemical test for iron metabolism and Next generation sequencing panel (HFE, HAMP, TF, TFR2, HJV, SLC40A1, BMP6, CP) were performed centrally.

Results:
119 patients (75.6% male, age 57years±13) were included. 44 patients had C282Y/H63D compound HFE heterozygosity and 16 were C282Y HFE heterozygotes. Ferritin levels were 1417µg/L±1417, transferrin saturation was 60.3%±19.2, LIC was 200µmol/g±108. Mean hepcidin/ferritin ratio was 2±1.4, significantly lower than in healthy volunteers. Non transferrin bound iron (NTBI) was present in 98 patients (mean=1.25µmol/L±0.9) and LPI was present in 76 patients (mean=0.23µmol/L±0.4). LPI was significantly correlated to hepcidin/ferritin ratio, LIC and liver enzymes.
25 patients had definite diagnosis (10 HFE related and 15 non HFE related), 27 had variant of unknown significance of which had 19 putative digenic hemochromatosis. Eventually 68 patients had molecularly undefined hemochromatosis. Patient with definite diagnosis had significantly higher ferritin, LIC, NTBI and LPI.

Conclusion:
Our results suggest that current genetic panel testing methods yields definite diagnosis in only half of patients with non HFE c282y hemochromatosis, and that the hepcidin/ferritin ratio can be useful to assess the severity of hemochromatosis and confirm hepcidin deficiency.



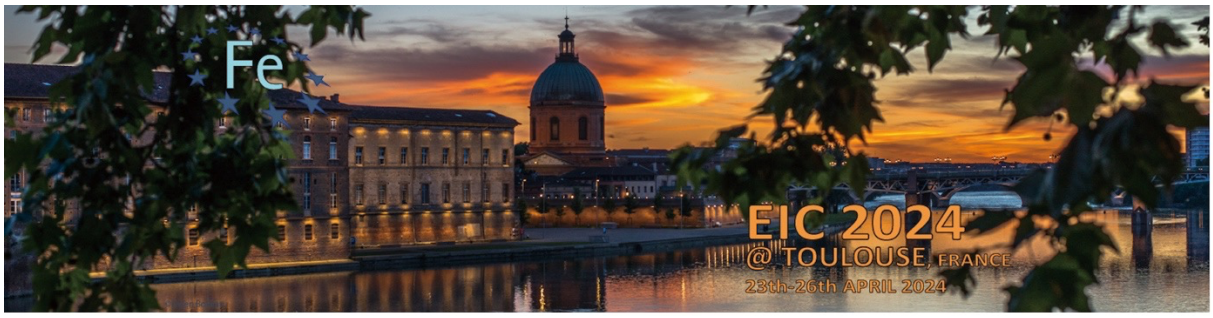
P-80 - BONE MARROW TFR2 DELETION COOPERATES WITH ACTIVIN LIGAND TRAP RAP-536 IN AMELIORATING β-THALASSEMIA

TANZI Emanuele [1,2], DI MODICA Simona Maria [1], BORDINI Jessica [2,3], OLIVARI Violante [1,2], PAGANI Alessia [1], FURIOSI Valeria [1], SILVESTRI Laura [1,2], CAMPANELLA Alessandro [2,3], NAI Antonella [1,2]

[1] Regulation of Iron Metabolism Unit, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan (Italy), Milano, Italy; [2] Vita-Salute San Raffaele University, Milan (Italy), Milano, Italy; [3] B-cell Neoplasia Unit, Division of Experimental Oncology, IRCCS Ospedale San Raffaele, Milan (Italy), Milano, Italy

β-thalassemia, caused by mutations in the β-globin gene, is characterized by anemia, ineffective erythropoiesis (IE), and iron overload. Current management of the disease is still suboptimal, with bone marrow transplantation and gene therapy being the sole curative options. Luspatercept, an activin receptor-ligand trap, is a promising novel approach which stimulates erythroid differentiation inhibiting the TGF-β pathway. However, its exact mechanism of action and the possible connection with erythropoietin remain to be clarified. Moreover, Luspatercept does not correct all the features of the disease. For these reasons, we investigated the effect of combining Luspatercept with hematopoietic transferrin receptor 2 (TFR2) inactivation, which stimulates erythropoiesis enhancing erythropoietin signaling,

To this aim, *Hbb*$^{th3/+}$ mice with or without hematopoietic *Tfr2* (*Tfr2*^{BMKO}/*Hbb*$^{th3/+}$) were treated with RAP-536 (the murine Luspatercept analogue) or vehicle. As expected, both Tfr2 deletion and RAP treatment improved anemia and IE in *Hbb*$^{th3/+}$ mice. Furthermore, hematopoietic *Tfr2* deletion, but not RAP-536, reduced spleen size and erythropoietin levels, and corrected iron-overload..Interestingly, *Tfr2* deletion and RAP-536 had an additive effect on improving anemia and IE, further reducing spleen size and liver inflammation,. Of note, RAP-536 efficacy in correcting anemia and IE was comparable in both *Hbb*$^{th3/+}$ and *Tfr2*^{BMKO}/*Hbb*$^{th3/+}$ animals, suggesting a TFR2 and erythropoietin-independent effect of the drug on erythroid differentiation. Our study sheds light on Luspatercept's mechanism of action,and suggests that strategies aimed at inhibiting TFR2 may enhance the therapeutic efficacy of activin receptor ligand-traps, ameliorating anemia, IE and iron overload in β-thalassemia, and potentially preventing severe complications.

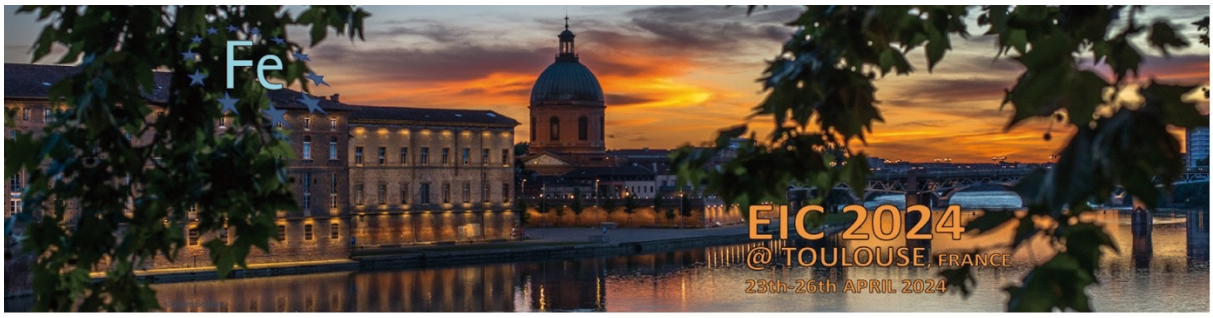


P-81 - TMPRSS6 INHIBITION IMPROVES BONE HEALTH IN B-THALASSEMIA MICE

CROWELL Beth [1], DAS Nanditha [1], IVANOVA Larisa [1], NANNURU Kalyan [1], ECONOMIDES Aris N [1], HATSELL Sarah J [1], LOB Heinrich [1]

[1] Regeneron Pharmaceuticals, Tarrytown, United States

Beta-thalassemia is a genetic disorder arising from mutations in the ß-globin gene, leading to ineffective erythropoiesis and iron overload contributing to liver fibrosis, cardiac toxicities and osteoporosis. The bone marrow expansion and iron overload, due to ineffective erythropoiesis, are the main drivers of reduced bone quality. To target the iron overload, we generated a monoclonal antibody (REGN7999) inhibiting TMPRSS6, a negative regulator of hepcidin, the main iron homeostasis regulating hormone. In the $Hbb^{th3/+}$ mouse model of ß-thalassemia, REGN7999 treatment reduced liver and serum iron and improved red blood cell health. Oxidative stress and senescence of red blood cells was reduced resulting in reduced red blood cell turnover and more effective erythropoiesis in ß-thalassemia mice. Using µCT, we tested if bone quality improves in response to TMPRSS6 inhibition, as erythropoiesis is now more effective and iron loading reduced. We found that bone mineral density and bone mineral content in ß-thalassemia mice were restored to wildtype levels after 8 weeks of REGN7999 treatment. Taken together, these data suggest that improving red blood cell health and reducing iron loading reverses the ß-thalassemia associated osteoporosis. This effect can potentially improve the quality of life of ß-thalassemia patients.

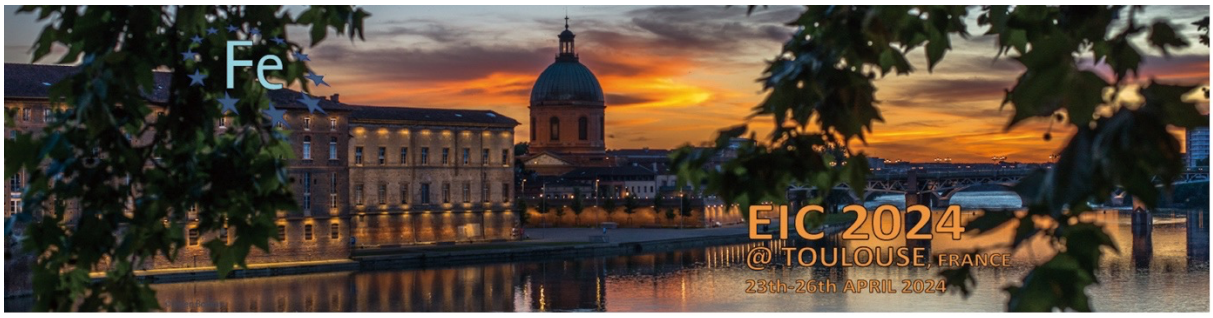


P-82 - Intestinal ferroportin protein expression in murine models of hemojuvelin overexpression

HORNUNG Isabelle [1], DOGAN Deniz [1], POPP Rüdiger [2], URZICA Eugen [3], OGUAMA David [3], FETTE Franca [1,3], NGUYEN Lien [3], LOICK Paul [1,3], RAUER Marcel [1,3], FLEMING Ingrid [2], ROSENBAUER Frank [4], ZACHAROWSKI Kai [1], SCHRADER Lisa [1,3], STEINBICKER Andrea [1,3]

[1] Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt am Main, Germany; [2] Department of Molecular Medicine, Institute of Vascular Signaling, Goethe University Frankfurt, Frankfurt am Main, Germany; [3] Department of Anaesthesiology, Intensive Care and Pain Medicine, University Hospital Muenster, University of Muenster, Muenster, Germany; [4] Institute of Molecular Tumor Biology, University Hospital Muenster, University of Muenster, Muenster, Germany

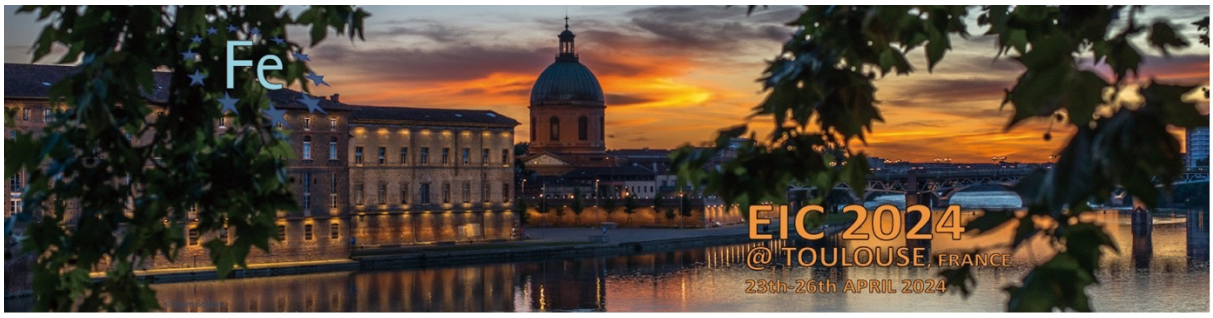
The hepatic hormone hepcidin is the key regulator of systemic iron homeostasis. It acts on intestinal iron absorption through the negative post translational regulation of the iron exporter ferroportin in enterocytes. Hepcidin expression is mainly regulated via the BMP/SMAD signaling pathway and induced by the BMP co-receptor hemojuvelin (HJV) under physiological conditions. In the current experiment, we investigated the interaction of HJV with the BMP type I receptor ALK3. We used an established model of adeno-associated virus (AAV) to overexpress HJV in hepatocyte-specific Alk3 deficient mice and appropriate controls. Mice with hepatocyte-specific ALK3 deficiency present with severe iron overload and hepcidin deficiency. The hepatocyte-specific Alk3 deficiency and HJV overexpression was confirmed by qPCR. Subsidiary, immunoblotting showed liver specific increased HJV in AAV-injected mice. In control mice, HJV overexpression led to increased hepatic Hamp mRNA levels. In contrast, in Alk3$^{fl/fl}$;*Alb-Cre* mice with AAV-HJV, hepcidin mRNA levels decreased to an even lower level. In addition, effects of HJV overexpression on ferroportin were determined in histological stainings. In the intestine of Alk3$^{fl/fl}$;*Alb-Cre* mice, ferroportin was increased compared to controls. Administration of HJV reduced ferroportin in Alk3$^{fl/fl}$ mice, while there was a slight increase of ferroportin in Alk3$^{fl/fl}$;*Alb-Cre* mice compared to respective PBS-injected controls. To determine erythropoiesis in the setting of HJV overexpression, bone marrow was analyzed via FACS. There was no change in erythropoiesis. To conclude, the data indicate that HJV mediates the activation of BMP/SMAD signaling via ALK3 *in vivo*.



P-83 - THE MAPK AND NRF2 PATHWAYS ACT IN CONCERT TO CONTROL IRON-INDUCED BMP6 EXPRESSION IN LIVER SINUSOIDA

QIU Ruiyue [1], CUCINELLI Stefania [1,2], COLUCCI Silvia [1,2,3], U. MUCKENTHALER Martina [1,2]
[1] Department of Pediatric Oncology, Hematology and Immunology and Hopp Children Cancer Center (KiTZ), University Hospital Heidelberg, Heidelberg, Germany; [2] Molecular Medicine Partnership Unit (MMPU), EMBL and Heidelberg University, Heidelberg, Germany; [3] European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Liver sinusoidal endothelial cells (LSECs) play a critical role in sensing systemic and/or liver iron levels and release bone morphogenetic proteins (BMPs) that control hepcidin levels in hepatocytes. Our previous results demonstrated that iron-dependent *Bmp6* induction in LSECs requires component(s) of the hepatocyte secretome (PMID: 36187873), but the exact mechanism(s) of how iron controls BMP6 levels remains unclear. Here we show that LSECs in iron-loaded *Fpn*(C326S) mice show activated PI3K-AKT and MAPK-ERK1/2 signaling pathways. Likewise, primary LSECs cultured in the presence of hepatocyte-conditioned medium with iron show increased phospho-ERK and phospho-AKT levels. Importantly, pretreatment of LSECs with MAPK signaling inhibitors targeting RAF, MEK1/2 and ERK1/2 (Raf265, U0126 and Ulixertinib, respectively), but not with the PI3K inhibitor Wortmannin, attenuated iron-induced *Bmp6* expression under hepatocyte-conditioned medium, suggesting the involvement of the ERK1/2 pathway in iron-mediated *Bmp6* control. Interestingly, the iron-induced *Bmp6* response was only completely abolished when the ERK1/2 pathway was inhibited together with the Nrf2 pathway, suggesting a collaborative function of the ERK1/2 and Nrf2 pathways in controlling BMP6 levels. Nrf2 is activated by pro-oxidant cellular states. Consistently, we show that H_2O_2 treatment of LSECs induces *Bmp6* mRNA expression, a response that was fully prevented by the ROS scavenger N-Acetyl-L-Cystein (NAC) in the presence of hepatocyte-conditioned medium. By contrast, NAC only attenuated iron-induced *Bmp6* mRNA levels, suggesting that iron in LSECs only partially activates *Bmp6* by generating oxidative stress. Additional experiments are ongoing to evaluate how the ERK1/2 and Nrf2 pathways interact in controlling the iron sensing process in LSECs.



P-84 - HYPOXIA DOWREGULATES HEPCIDIN AND UPREGULATES FGL1 EXPRESSION IN MICE

KRIJT Jan [1], BÁJECNÝ Martin [1], AHMED Fatima [1], TRUKSA Jaroslav [2], VOKURKA Martin [1]
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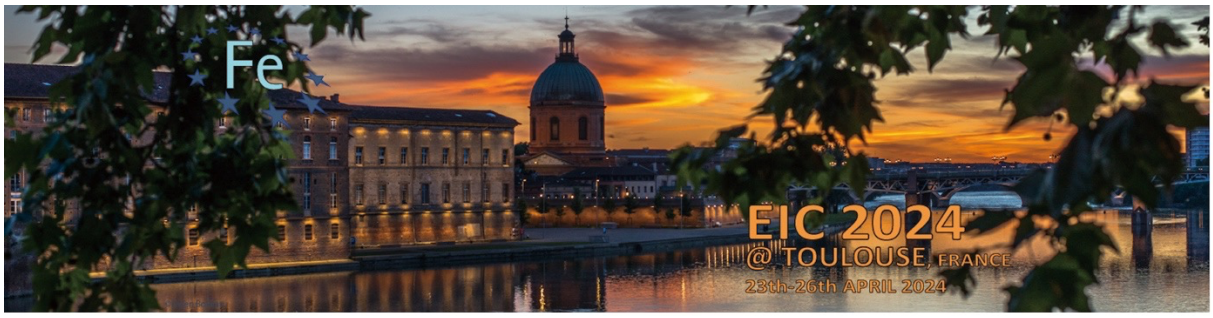
Repeated administration of erythropoietin (EPO) decreases liver hepcidin expression; the exact mechanism of this effect is unknown. Possible mediators include secreted proteins such as erythroferrone. Recently, it has been proposed that hepcidin downregulation by bleeding is partially mediated by the hypoxia-responsible hepatokine FGL1. The purpose of this study was to examine the response of *Fgl1* mRNA to hypoxia *in vivo*, and to determine whether FGL1 protein could contribute to hepcidin downregulation by EPO. Male C57BL/6J mice were subjected to 10% oxygen hypoxia for one week. The treatment increased hematocrit and spleen size; hepcidin expression decreased by about two PCR cycles. In addition, hypoxia increased *Fgl1* mRNA content (Δ CT 2.32 vs 3.08; n=6, $p<0.05$). These data support the possible role of FGL1 in hepcidin downregulation by hypoxia.
To examine the contribution of FGL1 to hepcidin suppression by EPO, groups of intact and splenectomized male C57BL/6J mice were administered 50 U of EPO for four days. Hepcidin dramatically decreased in intact mice (Δ CT 6.49 vs 0.25); in splenectomized mice, the decrease was much less pronounced (2.06 vs 0.13; n=5). *Fgl1* mRNA content was unaffected by EPO treatment. These results suggest that the main signal responsible for EPO-induced hepcidin downregulation in mice originates in the spleen. Both erythroferrone and FGL1 act by binding the BMP6 protein. However, EPO administration to female *Bmp6*-/- mice still markedly decreased hepcidin expression (9.15 vs 0.25). These data suggest that additional factors other than erythroferrone and FGL1 contribute to hepcidin downregulation by EPO in mice.



P-85 - PHARMACOKINETICS AND PHARMACODYNAMICS OF AN ANTI-HEMOJUVELIN MONOCLONAL ANTIBODY IN MICE

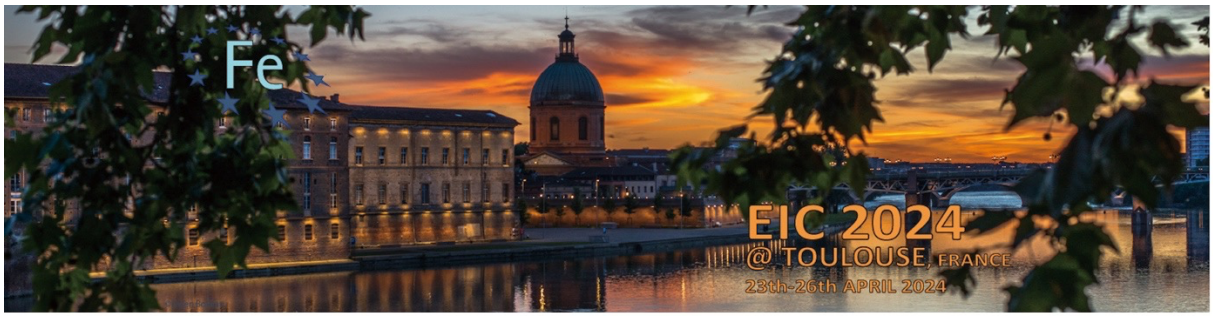
XU Julia [1], MACDONALD Brian [1], **WU Min [1]**
[1] DISC Medicine, Watertown, United States

Hemojuvelin (HJV/RGMc) is a BMP co-receptor that specifically regulates hepcidin gene expression. Hepcidin plays a major role in iron homeostasis. High hepcidin levels inhibit intestinal iron absorption and macrophage iron recycling, causing iron-restricted erythropoiesis and anemia. Conversely, low hepcidin levels lead to an increase in iron availability for enhanced erythropoiesis.

DISC-0974 is a humanized anti-hemojuvelin (HJV) monoclonal antibody designed to block the interaction between HJV and bone morphogenetic protein (BMP) receptors leading to decreased hepcidin expression. DISC-0974 is currently in clinical trials to treat patients with anemia of myelofibrosis, and patients with NDD-CKD and anemia.

A murine analog of DISC-0974, PRO-1535, was developed to avoid immunogenicity in mouse studies. To evaluate the PK PD effects of PRO-1535, male C57/BL6 mice were dosed once with vehicle or PRO-1535 at 0.2, 2, or 20 mg/kg intravenously. Blood and liver samples were collected on days 1, 3, 7, and 10 post-dosing. PRO-1535 administration was well-tolerated. Dose proportional increase in PRO-1535 concentration in plasma was observed in treated mice. With respect to PD, PRO-1535 decreased serum hepcidin level, and its liver mRNA expression in a dose-dependent manner. Furthermore, PRO-1535 administration was associated with increased serum iron and TSAT.

Taken together, this study demonstrated that PRO-1535, a mouse anti-HJV monoclonal antibody, can inhibit hepcidin production and enhance serum iron level in vivo. PRO-1535 can be used in efficacy studies in mouse disease models and further support the therapeutic potential of DISC-0974 for the treatment of anemia.



P-86 - A PHASE 1B TRIAL OF DISC-0974, AN ANTI-HEMOJUVELIN ANTIBODY, IN PATIENTS WITH MYELOFIBROSIS AND ANEM

GANGAT Naseema [2], **WU Min [1]**, FORAN James [3], HALPERN Anna [4,5], RAMPAL Raajit [6], BOSE Prithviraj [7], BHATT Sima [1], BUCH Akshay [1], PELLETIER Olivia [1], SAVAGE William [1], TEFFERI Ayalew [2]

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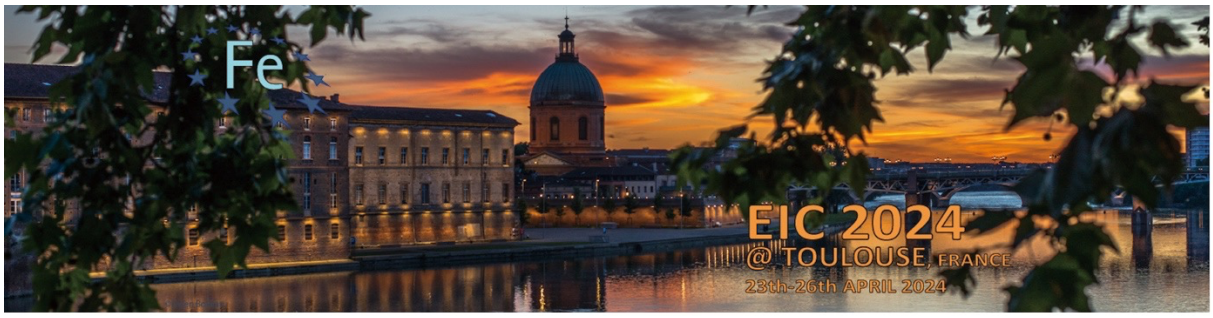
Hepcidin, a central regulator of iron homeostasis, is pathologically elevated in patients with myelofibrosis (MF) and anemia. Chronic elevations in hepcidin contribute to the onset and severity of anemia. DISC-0974 is an investigational, first-in-class, monoclonal antibody that blocks hemojuvelin, a co-receptor in the bone morphogenetic protein-signaling pathway driving hepcidin expression. A healthy volunteer study has demonstrated dose-dependent reductions in serum hepcidin, increases in serum iron with doses up to 56 mg administered subcutaneously, and increasing trends in reticulocyte count, reticulocyte hemoglobin, mean corpuscular hemoglobin, total hemoglobin (Hgb), and red blood cell count.

In this Phase 1b/2a, open-label, multiple-ascending dose study (NCT05320198), we are assessing the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of DISC-0974 in patients with MF and anemia.

Eligible participants include patients over 18 years of age with intermediate-2 or higher-risk MF and anemia. MF is required to be confirmed using the World Health Organization 2016 criteria. Anemia is defined per protocol as Hgb < 10 g/dL or transfusion dependence, as defined by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). A stable dose of hydroxyurea and/or Janus kinase (JAK) inhibitor is allowed on trial.

Primary endpoints include safety and tolerability assessments of adverse events (AEs), clinical laboratory assessments, vital signs, physical examinations, and electrocardiograms.

As of August 1, 2023, enrollment and dose escalation are ongoing. Safety, PK and PD data from at least the first three cohorts will be presented.



P-87 - PIEZO1 GAIN-OF-FUNCTION VARIANTS REGULATE HEPCIDIN EXPRESSION BY MODULATING mTOR/LAMTOR4 SIGNALLING

ROSATO Barbara Eleni [1,2], CARLEO Rossana [3], D'ONOFRIO Vanessa [1,2], ESPOSITO Federica Maria [1,2], PETTINATO Mariateresa [3], PAGANI Alessia [3], MARRA Roberta [1,2], CAPASSO Mario [1,2], IOLASCON Achille [1,2], RUSSO Roberta [1,2], SILVESTRI Laura [1,2], ANDOLFO Immacolata [1,2]

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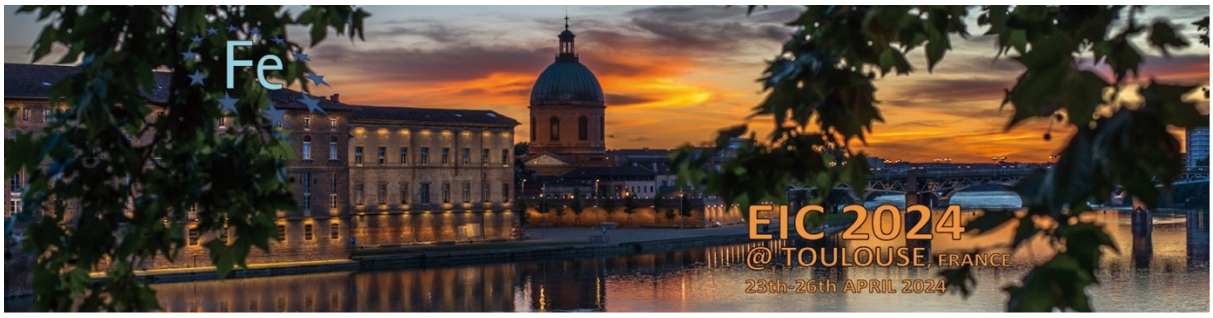
PIEZO1 is a mechanoreceptor that plays a crucial role in various biological processes. Gain-of-function (GoF) variants in PIEZO1 cause dehydrated hereditary stomatocytosis (DHS), a pleiotropic syndrome characterized by anemia and transfusion-independent iron overload. Interestingly, the expression of hepcidin, the master regulator of iron metabolism, is reduced in DHS patients and in PIEZO1 mutant cells via an unknown mechanism.
To learn more about the mechanism, we developed a human Hep3B cell line expressing the PIEZO1-R2456H variant and performed transcriptomic and proteomic analyses. Interestingly, we found that the mTOR signaling is one of the pathways most affected by PIEZO1-R2456H. We show that the expression of Late Endosomal/Lysosomal Adaptor, MAPK And mTOR Activator 4 (LAMTOR4) is strongly downregulated in PIEZO1-R2456H cells. The Ragulator complex plays a crucial role in the mTOR pathway by binding mTORC1 to the lysosome. Since mTORC1 is closely linked to the BMP-SMAD pathway, we knocked down Lamtor4 in murine primary hepatocytes and analyzed hepcidin and the BMP-SMAD pathway. We found that autophagy is increased in the absence of Lamtor4. In parallel, hepcidin is downregulated, likely due to inhibition of the BMP-SMAD pathway, being Id1 also downregulated. However, siLamtor4 hepatocytes properly modulate BMP-SMAD signaling in response to activators or inhibitors, suggesting that LAMTOR4 is upstream of BMP receptors. Further studies are underway to better decipher the mechanism. Exploring the PIEZO1/LAMTOR4/mTOR axis as a novel player in modulating hepcidin expression will be useful to understand the pathogenic mechanism of DHS and identify novel drug targets to alleviate DHS-associated iron overload.

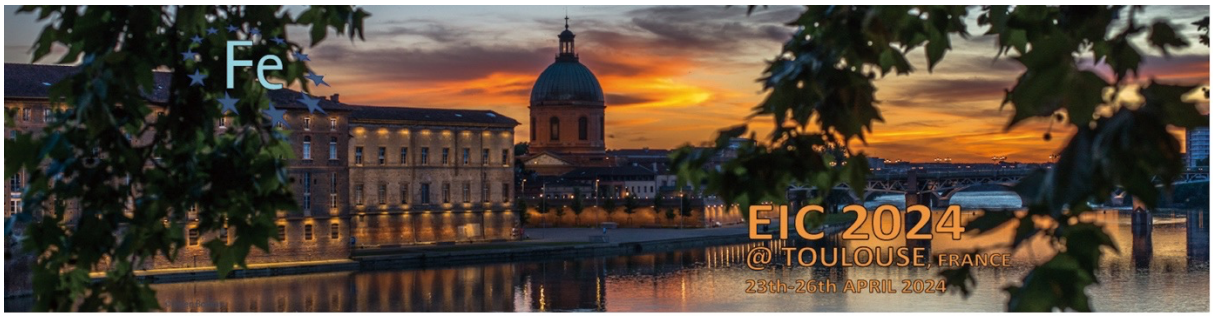


P-88 - THE INFLUENCE OF DIETARY CONTAMINATION WITH AFLATOXIN AFB1 ON BASIC INDICATORS OF IRON METABOLISM

OGLUSZKA Magdalena [1], STARZYNSKI Rafal [2], KOZERA Wojciech [3], LEPCZYNSKI Adam [4], SLASKA Brygida [5], TANIGUCHI Hiroaki [6], LIPINSKI Pawel [2], KEPKA Katarzyna [1], OZGO Malgorzata [4], HEROSIMCZYK Agnieszka [4], MARYNOWSKA Marta [4], TKACZYK-WLIZLO Angelika [5], GRYCHNIK Pawel [5], POLAWSKA Ewa [1], PAREEK Chandra [7], PIERZCHALA Mariusz [1]

[1] Department of Genomics, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, Poland; [2] Department of Molecular Biology, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, Poland; [3] Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; [4] Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology, Szczecin, Poland; [5] Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Lublin, Poland; [6] Department of Experimental Embryology, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, Poland; [7] Institute of Veterinary Medicine, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, Torun, Poland

Fungal mycotoxins, common food contaminants, contribute to food losses. Particular the aflatoxins (AF) B1, B2, G1 and G2 synthesized by Aspergillus sp. have an adverse effect on human and animal health, including the development of hepatotoxicity and cancer. Cases have been reported in which a single administration of AF resulted in changes in clinical outcomes, serum biochemistry, histology, and hematology. In our experiment, aflatoxicosis was studied in 24 4-week-old Polish Landrace piglets from 6 litters, randomly assigned to 3 experimental groups (N = 6) receiving: 0, 30 and 60 µg AFB1/kg b.w. for 28 consecutive days. The animals were slaughtered, weighed and samples taken for analysis. AFB1 administration had no effect on feed intake, daily gain and red blood cell indices (Hb, RBC, MCV, MCV, Ret) in all groups. Statistically significant differences were observed in the WBC count in the group receiving 60 µg AFB1/kg b.w. compared to the other groups. The analysis of the content of non-heme and heme iron in tissues and plasma iron did not change, although an increasing trend was observed in the group of piglets receiving the highest dose of AFB1. Similarly, the highest dose resulted in nonsignificant but higher levels of nonheme iron in duodenal scrapings. Preliminary conclusions indicate that low concentrations of AFB1 do not disrupt iron homeostasis, although this dose confirms a complex, direct toxic effect causing neutrophil mobilization. Promising studies are currently underway with an actual feed/food contaminating dose of 120 µg AFB1/kg bw.

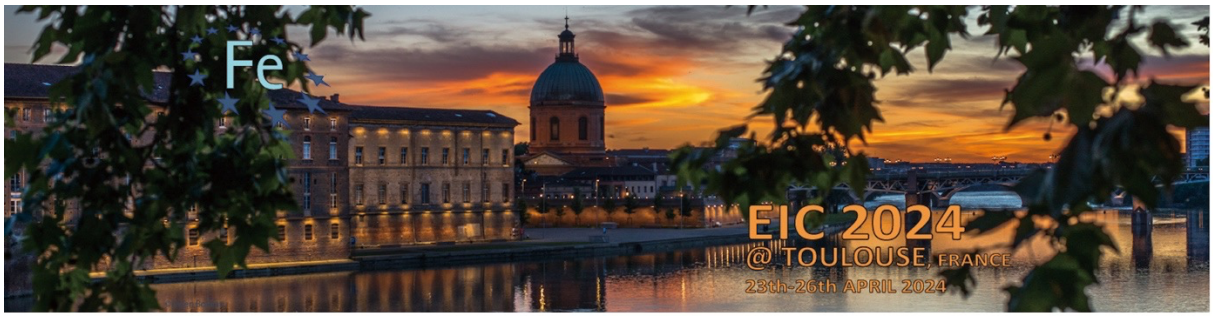


P-89 - UNRAVELLING THE ROLE OF SUV420H2 IN A MOUSE MODEL OF OBESITY, INSULIN RESISTANCE, AND T2DM

CARLEO Rossana [2], BAVUSO VOLPE Letizia [2], PETTINATO Mariateresa [2], FURIOSI Valeria [2], NAI Antonella [1,2], PAGANI Alessia [2], SILVESTRI Laura [1,2]

[1] Vita-Salute San Raffaele University, Milan, Italy; [2] Regulation of Iron Metabolism Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy

Dysregulation of iron metabolism is associated with various metabolic diseases, such as Metabolic dysfunction-associated fatty liver disease (MAFLD), insulin resistance (IR) and Type 2 diabetes (T2DM). Conversely, weight gain and total body fat distribution influence iron metabolism. A common association between liver iron and liver triglycerides levels has been mapped on a region of chromosome 7 that contains the histone methyltransferase Suv420h2. Interestingly, Suv420h2 deletion, in conjunction with Suv420h1 downregulation, inhibits Ppar, a key transcription factor involved in glucose and lipid metabolism, and counteracts diet-induced obesity.
We then decided to dissect the role of these methyltransferases in a mouse model of obesity, insulin resistance, and T2DM, the db/db mice, focusing on Suv420h2. Db/db mice recapitulate the T2DM main features, displaying an obese and steatotic profile characterized by a substantial increase in body weight and adipose tissue, as well as insulin resistance and hyperglycemia. Hepatomegaly is associated with decreased liver iron concentration and elevated serum iron levels, indicating deregulated hepcidin function. Interestingly, deletion of Suv420h2 in db/db leads to decreased body weight, reduced white adipose tissue hypertrophy and a trend towards a decrease in hepatomegaly. This is accompanied by decreased serum iron levels and increased liver iron concentration. Hyperglycemia is also improved while IR is not affected by the deletion of Suv420h2. Overall, these data suggest that global Suv420h2 deletion counteracts obesity and partially improves T2DM but does not correct IR. Further studies are underway to fully characterize the mechanism(s) involved.



P-90 - Hepatocyte-specific inactivation of the epigenetic regulator SUV420H1 improves MASLD in mice

BAVUSO VOLPE Letizia [1], PETTINATO Mariateresa [1,2], FURIOSI Valeria [1], CARLEO Rossana [1], NAI Antonella [1,2], SILVESTRI Laura [1,2], **PAGANI Alessia [1,2]**

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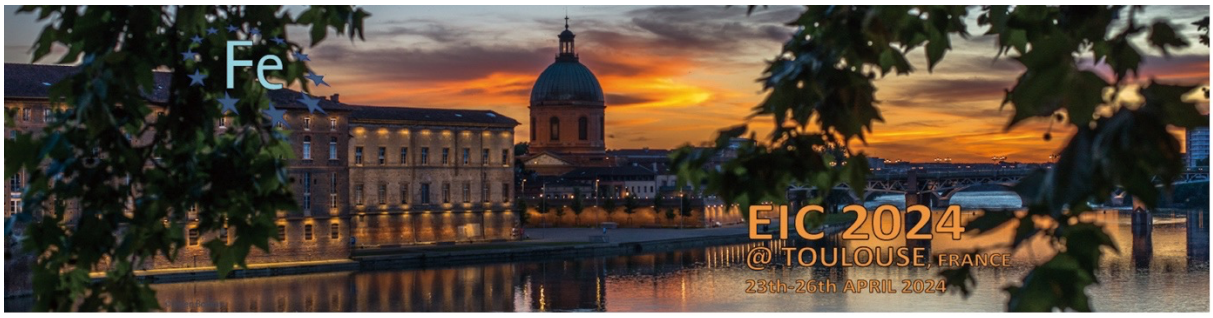
Metabolic-dysfunction-Associated-Steatotic-Liver-Disease (MASLD) is the hepatic manifestation of the metabolic syndrome characterized by dyslipidemia, insulin resistance, liver inflammation and fibrosis. MASLD is influenced by nutritional, environmental, and genetic factors. The epigenetic regulators Suv420h1-h2 have been identified as inhibitors of Ppar signaling in brown adipose tissue. Recently, a GWAS performed on mice identified a common association between liver iron and triglyceride levels in a region of chromosome 7 containing, among others, Suv420h2. We have previously shown that mice with Suv420h1-Suv420h2 deletion are protected from MASLD. However, their specific role in disease progression is unknown. To investigate whether Suv420h1 can protect against MASLD, we generated mice lacking h1 only in hepatocytes (h1-LCKO) that were fed a fructose-palmitate-cholesterol-rich diet (FPC) for 16 weeks. FPC-h1-LCKO mice gain less weight compared to FPC-control mice. Glucose levels are lower and insulin sensitivity improves in the absence of h1. Hepatomegaly, liver triglyceride content and lipid droplets are reduced in h1-LCKO mice. Interestingly, the expression of genes associated with FPC-induced inflammation and fibrosis is comparable to that of FPC-control mice. However, fibrosis measured with Sirius Red is lower, and accordingly, the marker Bmp8b associated with disease severity is also greatly reduced in h1-LCKO mice. Of note, the expression of Ppar-alpha is increased in the liver of FPC-h1-LCKO mice, suggesting that it may be involved in disease amelioration. Overall, these data show that hepatocyte-specific deletion of Suv420h1 plays a protective role in diet-induced MASLD and complications. Further studies are ongoing to identify the pathways modulated by Suv420h1 in the liver.



P-91 - STEATOSIS CAUSES HEPATIC IRON RESISTANCE IN TYPE 2 DIABETES.

ALTAMURA Sandro [1], MUDDER Katja [1], SACCHI Chiara [2], QIU Ruiyue [1], WIRTH Angela [3], FREICHEL Marc [3], MUCKENTHALER Martina [1]

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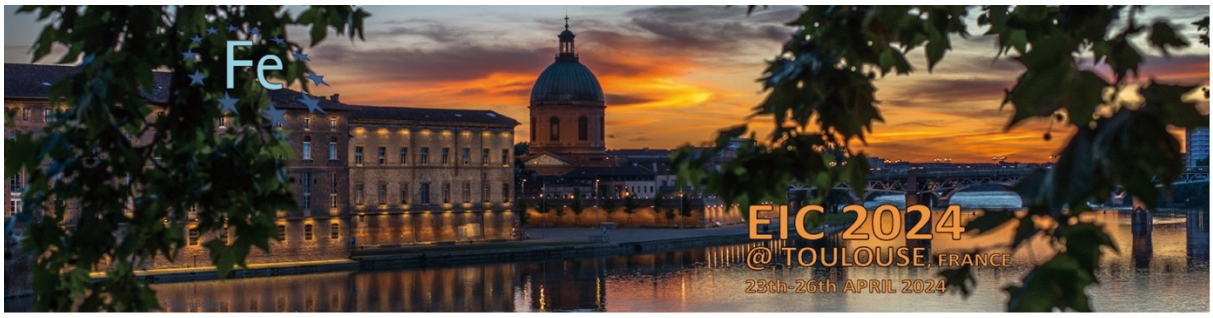
Type 2 diabetes mellitus (T2DM) is a metabolic disorder hallmarked by hyperglycaemia due to insulin resistance, increased body mass index and hepatic steatosis.
We have previously demonstrated that T2DM patients show inappropriately low hepcidin levels despite increased systemic iron. Analysis of the db/db mouse model of T2DM further showed an “iron resistance” phenotype, whereby the steatotic liver is iron deficient in the presence of increased plasma iron levels.
We now aim to identify the mechanism(s) how T2DM causes hepatic iron resistance. We excluded hyperglycaemia and reduced insulin-related signalling, since iron-parameters remained unaltered in non-obese insulin-deficient Ins2(Akita) mice. We next generated the western diet-induced model of NAFLD. These mice show normoglycemia and unaltered expression of key genes involved in glycolysis and gluconeogenesis compared to wild-type mice. In this model, the liver is steatotic and iron deficient while plasma iron levels are elevated, mirroring T2DM. Hepcidin mRNA expression is strongly decreased even though mRNA expression of other BMP/SMAD target genes is unaltered. To identify the mechanism through which hepatic lipid accumulation causes “iron resistance”, we established an in vitro model of steatosis: Huh-7 cells treated with palmitic and oleic acids become “steatotic”. In this model, the overall TfR1 protein levels are unchanged, but its cell surface expression is increased. Despite this, the binding affinity of TfR1 for transferrin is reduced. These results suggest that steatosis affects TfR1 function thus causing iron resistance, suggesting a new role for TfR1 in mediating increased systemic iron levels in T2DM patients.



Index