V JORNADA DE JÓVENES INVESTIGADORES DEL IMIBIC

HOSPITAL UNIVERSITARIO REINA SOFÍA
ASSEMBLY HALL
MAY 6 2014, CORDÓBA

Abstract book

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Distribution:

Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)
Hospital Universitario Reina Sofía
Edificio Consultas Externas, Nivel -1 Avda. Menéndez Pidal, s/n Córdoba
14004
www.imibic.org

2014 Edition

Thank-you notes:
The coordinators of the IMIBIC's V Jornada de Jóvenes Investigadores would like to express their sincere gratitude to all the researchers who have been members of the scientific committee this edition. With no doubt, their effort and advice raise the standards of this meeting year by year.
Moreover, they would like to thank Colegio de Médicos de Córdoba for its commitment on the promotion of the research activity among the medical residents

PROGRAMME

9:00-9:30	**Inscriptions and Posters display**
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10:00-11:00	**SESSION I. Infectious and immunological diseases. Organ transplantation. Active ageing and fragility**
I.a 10:00-10:15	External european validation of a multicenter model for donor-recipient matching in liver transplantation based on artificial neural networks. **Mª Dolores Ayllón**
I.b 10:15-10:30	Postsurgical drainage fluid as predictor marker of surgical-site infection after cervical neck dissection. **Alberto Candau Álvarez**
I.c 10:30-10:45	Lack of consensus for defining clinical suspicion of rejection after liver transplantation: Implications on clinical practice and possible solutions. **Manuel Rodríguez Perálvarez**
I.d 10:45-11:00	Acceptability and feasibility of the opportunist search of patients with HIV infection in primary care centers of the national health service. **Rafael Carlos Puentes Torres**
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II.b 11:45-12:00I	Testosterone induces transgenerational impairments on the behavior of C. elegans. A model to explain androgen-dependent autistic traits. **Mª del Mar Gámez del Estal**
II.c 12:00-12:15	Functional validation of SNP rs6105269 in Crohn's disease. **Patricia Aguilar Melero**
II.d 12:15-12:30	Inflammation impairs regulation of FGF23 by phosphorus in renal failure. **María Encarnación Rodríguez Ortiz**
II.e 12:30-12:45	Transcriptional analysis reveals different immune regulation between scalp and skin psoriasis with potential therapeutic implications. **Juan Ruano Ruiz**
II.f 12:45-13:00	Effects of calcitriol and paricalcitol on osteogenesis of bone marrow mesenchymal stem cells. **Carmen Herencia Bellido**
13:00-14:00	**Plenary conference. Dr. José Luis García Pérez**
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16:00–17:15	**SESSION III. Cancer (Oncology and Oncohematology)**
III.a 16:00–16:15	Hypoxia Response Induces the Formation of Polyploid Giant Cells and Cancer Stem Cells in Colon Cancer. **Laura M. López Sánchez**
III.b 16:15–16:30	Lack of endogenous cortistatin but not somatostatin exacerbates carcinogen-induced mammary gland tumorigenesis in mice. **Alicia Villa Osaba**
III.c 16:30–16:45	The fungal metabolite galiellalactone blocks cell cycle progression in prostate cancer cells through STAT3 and NF-kB independent pathways. **Víctor García González**
III.d 16:45–17:00	In vivo effect of 5-azacitidine treatment in regulatory t cells in patients with aml / mds. **Diana Buenasmañanas Cervantes**
III.e 17:00–17:15	Modern surgical liver resections offer better results than transarterial chemoembolization in cirrhotic patients with b-bclc stage hcc. **Ana Belén Gallardo Herrera**
17:15–17:45	**Poster session**
17:45–19:00	**SESSION IV. Nutrition, Endocrine and metabolic diseases**
IV.a 17:45–18:00	SEPT11: a novel adipocyte marker related to obesity and insulin resistance. **Natalia Rocío Moreno Castellanos**
IV.b 18:00–18:15	Expression of Mkrn3 and mir-30b in the hypothalamus during postnatal maturation and in preclinical models of altered puberty: A novel regulatory system in the control of puberty onset? **Violeta Heras Domínguez**
IV.c 18:15–18:30	Levels of fitness and physical activity influence changes on response of plasma adipokines at prepubertal stage. **Francisco Jesús Llorente Cantarero**
IV.d 18:30–18:45	Beneficial Effect of CETP Gene Polymorphism rs3764261 in Combination with a Mediterranean Diet on Lipid Metabolism in the Patients with Metabolic Syndrome. **Ruth Blanco Rojo**
IV.e 18:45–19:00	Genetic targets which could determine the shortening of telomeres in obese people. **Oriol Rangel Zúñiga**
19:00–19:15	**Award-giving and Closing ceremony**

SESSION I

Infectious and immunological diseases
Organ transplantation
Active ageing and fragility

l a External european validation of a multicenter model for donor–recipient matching in liver transplantation based on artificial neural networks.

Authors: *Mª Dolores Ayllon, Rubén Ciria, Roberto Valente, Manuel Cruz-Ramírez, María Pérez-Ortiz, César Hervás, Mohammed Rela, Manuel de la Mata, John O'Grady, Nigel Heaton, Javier Briceño.*
Group: GC2 *Oxidative and Nitrosative Stress in Acute and Chronic Liver Disease*

Background and aims. Donor–recipient matching (D-Rm) remains a difficult clinical skill. We designed a pioneer model based on artificial-neural-networks (ANN) by a multicenter Spanish study (MADRE=Model for the Assignment of Donor and Recipient in Europe). The aim was to perform an external validation of MADRE with a high-volume European centre.

Materials: Retrospective analysis including 1437 D-R pairs (615-Spanish (11 hospitals) 2007-2008 and 822-British [King`s College Hospital-London] 2002-2010). Two reciprocal models (training MADRE-testing King's and vice versa) were performed. A third combined model was also created. For each pair, 38 "a-priori" donor, recipient and surgical variables were collected. Two non-complementary 3-months and 1-year endpoint models of probability-of-graft-survival (CCR) and probability-of-non-graft-survival (MS) were developed. Randomization was performed by a 10-fold. Comparisons with MELD, D-MELD, DRI, SOFT, P-SOFT and BAR were performed. WEKA machine was used for ANN calculations.

Results: From the 1437 D-R pairs, baseline significant differences among Spanish and King's pairs were observed in BMI (29.9 vs 32.6), HCV (24.7 vs 18.9), MELD (19.2 vs 18.6), donor-AST (55.4 vs 63.8), partial grafts (3.9% vs 9.1%) and cold ischaemia time (7.4 vs 8.8). Probability of 3-months and 1-year survival (CCR Model) was 90.28% and 87.5%, respectively. Probability of 3-months and 1-year not-survival was 64.29 and 61.34%, respectively. Both had excellent AUROC (0.824 and 0.793), which were statistically higher than other scores. A D-Rm proposal was performed by the ANN.

Conclusions. Our ANN-based model of D-Rm is the most advanced tool to date. Improved outcomes could be obtained by this technology.

I b Postsurgical drainage fluid as predictor marker of surgical-site infection after cervical neck dissection.

Authors: *Candau-Alvarez A, Dean-Ferrer A, Gil-Campos M, De la Torre-Aguilar MJ, Heredero-Jung S, Linares-Sicilia MJ, Perez-Navero JL.*
Group: GE2 *Child Metabolism*

Background: Surgical-site infection (SSI) after cervical neck dissection (CND) increases mortality, morbidity, length of stay and delays adjuvant treatment. The prevalence of SSI in head and neck squamous cell carcinoma (HNSCC) surgery varies from 10% to 40%. We present a novel protocol of study of the postoperative drainage fluid for an early SSI diagnosis by the determination of the cytokine profile changes and the bacteriological growth.

Methods: A prospective cohort study of 40 patients undergoing major head and neck oncological surgery was conducted. Drainage fluid samples were collected on days +1, +3, +5 and +7 after surgery. Microbiological routine cultures of the samples were performed. Determination of Interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-10 and Tumor Necrosis Factor-α (TNF-α) at days +1 and +3 were done by ELISA. Patients were followed-up to detect SSI following the standard clinical criteria of the Center for Disease Control (CDC).

Results: SSI was diagnosed in 6/40 patients. Routine culture diagnostic profitability on postoperative day +3 runs as follows: sensitivity (Se)=83.33%, specificity (Sp)=73.53%, positive predictive value (PPV)=35.71%, negative predictive value (NPV)=96.15%. Diagnostic profitability on postoperative day +5 was of Se=100%, Sp=66.66%, PPV=37.5% and NPV=100%. IL-2 and TNF-α did not show statistically differences among SSI and non-SSI patients. However, IL-1β, IL-6, IL-8 and IL-10 showed a constant pattern that can discriminate SSI and non-SSI patients at days +1 and +3 ($p<0.05$).
Conclusion: Assessment of drainage fluid cytokine levels in early postoperatory of CND may provide a novel method to early detection of SSI. Negative culture of drainage on postoperative day +3 predicts the nonappearance of SSI. This new protocol may allow clinicians to stratify those patients at higher or lower risk of SSI for a better resources assignment, avoid unnecessary antibiotic treatment and early therapeutical decision making to reduce mortality, morbidity and costs in addition.

I c Lack of consensus for defining clinical suspicion of rejection after liver transplantation: Implications on clinical practice and possible solutions.

Authors: *M. Rodríguez-Perálvarez, C. García-Caparrós, G. Germani; R. Ciria; Guerrero M, A. Poyato; JL. Montero; J. Briceño, J. O'Beirne, P. Barrera Baena, D. Patch; P. Burra; AK. Burroughs, M. De la Mata.*
Group: GC2 *Oxidative and Nitrosative Stress in Acute and Chronic Liver Disease*

BACKGROUND/AIMS: The lack of consensus to select patients for liver biopsy after liver transplantation (LT) would lead to heterogeneity in clinical practice and misdiagnosis of acute rejection (ACR). We aimed 1st) to evaluate the physician agreement to select patients with clinical suspicion of ACR, and 2nd) to design a model to predict moderate-severe histological ACR after LT.

METHODS: We randomly selected 100 LT patients from the Royal Free Hospital (1997-2007) who had a protocol liver biopsy between days 7-10 after LT to diagnose ACR. The clinical information between LT and protocol biopsy was given to 9 clinicians from 3 European transplant centres who decided if a liver biopsy was needed. The concordance among clinicians and with the histological evaluation (gold standard) was evaluated by the κappa coefficient. A multiple logistic regression model was designed to predict moderate-severe histological ACR.

RESULTS: Moderate-severe histological ACR occurred in 42 patients in the protocol biopsy evaluation (42%). The agreement among clinicians to select candidates for liver biopsy was poor: κ=0.06-0.62, being κ<0.40 in 76% of comparisons. The concordance between the indication of liver biopsy and moderate-severe ACR in the protocol biopsy was κ<0.30 in all cases. The so called "REJECTEST" model was composed by the following variables: Product of age by pre-LT MELD (OR=0.81; p=0.013); rising blood eosinophil count in the last 4 days prior to liver biopsy (OR=1.50; p=0.002); and reduced immunosuppression (OR=11.4; p=0.047) defined as mean trough concentrations of tacrolimus <6 ng/mL without other immunosuppressants. The area under ROC curve was 0.83. A total of 79.1% of patients were correctly classified by the "REJECTEST" model.

CONCLUSION: The agreement between clinicians to select patients for liver biopsy is very poor. The "REJECTEST" model allows for a uniform evaluation of ACR early after LT, and may benefit both clinical practice and randomized trial design.

I d Acceptability and feasibility of the opportunist search of patients with HIV infection in primary care centers of the national health service.

Authors: *Puentes Torres, Rafael; Aguado Taberné, Cristina; Castro Fernández, Cristina, Espejo Espejo, José; Pérula De Torres, Luis Ángel; Grupo Colaborativo estudio VIH-AP*
Group: GC12 *Investigación epidemiológica en Atención Primaria*

OBJETIVES: To check the acceptability and feasibility of opportunistic search of HIV infection in patients attending primary care centers.
MATERIALS AND METHODS. Observational, descriptive, cross-sectional study , approved by the ethics committee of clinical research of Cordoba. 52 doctors from 42 different health centers from all over Spain. We recluted patients between 18 and 65 years old needing blood test for other reasons and who had never been tested for HIV status. Variables: age, sex, tobacco/alcohol consumption, steady relationship, educational level, main reason for the blood test, acceptability of HIV test, main reason for refusing test/for not to have done HIV test previously, and test result.
RESULTS: 91'5% accepted HIV test. 52'5% of them had never done it before considering not to be at risk and 43'1% said their doctors had never offered them the test. 8'5% did not accept HIV serology. 59'6% of them considered not to be at risk and 21'1% were afraid of a positive result. We observed a linear relation with greater acceptability to higher levels of education ($p<0'05$). No significant differences in other variables were observed. The incidence of HIV + was 0'5%.
CONCLUSIONS: Offering HIV test as an opportunistc search in primary care is accepted by patients and feasible by doctors as well as profitable (HIV+ >0'1%).

SESION II

Chronic and Inflammatory diseases

II a Charaterization of MicroRNAs involved in the regulation of atherotrhombosis in Antiphospholipid Syndrome and Systemic Lupus Erythematosus

Authors: *Perez-Sanchez C,Ruiz-Limon P, Teruel R, Aguirre MA, Carretero-Prieto RM, Barbarroja N, Jimenez-Gomez Y, Rodriguez-Ariza A, Collantes-Estevez E, Gonzalez-Conejero R, Martinez C, Cuadrado MJ and, Lopez-Pedrera Ch.*
Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Background: miRNAs are key players in pathophysiological processes, but no previous studies have investigated their asscociation with the cardiovascular and atherothrombotic risks observed in primary antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE).
Objective: To characterize the miRNAs involved in pro-inflammatory, prothrombotic and pro-oxidative status in SLE and APS patients.
Methods: In silico search was performed and six putative miRNAs regulating factors involved in the proinflammatory and pro-oxidative status of APS and SLE patients (miR-124a, -125a, -125b, -146a, -155, and -222) were selected and quantified by RT-PCR in neutrophils, lymphocytes and monocytes from 15 APS and 22 SLE patients, and 26 healthy donors. Pro-inflammatory and prothrombotic proteins and oxidative stress markers were evaluated by flow cytometry. Proteins related to the biogenesis of miRNAs were quantified by RT-PCR and Western Blot.
Results: Expression of selected miRNAs was significantly decreased in neutrophils from SLE and APS patients compared to healthy donors. However, only miR-124a was found significantly reduced in monocytes from SLE and APS patients. No changes were found in lymphocytes. The expression of those miRNAs negatively correlated with autoimmunity-related parameters (anti-dsDNA and aCL-IgG), disease activity (SLEDAI), inflammation (IL-2, -6, -8 and MCP-1) and oxidative stress (peroxides). Low levels of specific miRNAs in neutrophils and monocytes from both SLE and APS were found further associated with thrombotic events and pathological carotid intima media thickness. This selective decrease miRNA expression was related to a significant reduction in the expression of miRNAs biogenesis genes (Xportin-5, Drosha, Dicer, Ago-1 and Ago-2) in neutrophils from APS and SLE patients compared to healthy donors. In vitro treatment of healthy monocytes with purified antiocardiolipin antibodies from APS patients caused a significant decrease in the levels of miR-124a. Notably, when this miRNA was transfected into monocytic THP-1 cells a significant decrease (~30%) in MCP-1, STAT3 and p38 expression was observed, pointing at these proteins as potential targets of miR-124a.
Conclusion: 1. Decreased expression of a number of miRNAs in monocytes and neutrophils from APS and SLE patients correlates with autoimmunity, inflammation, thrombosis and oxidative stress and atherothrombotic markers. 2. A down-regulation of miRNA processing might explain the low expression of evaluated miRNAs in APS and SLE neutrophils. 3. Antiocardiolipin antibodies directly regulate miR-124a expression, which is directly involved in the proatherosclerotic profile of APS and SLE patients.
Supported by: P08-CVI-04234, CTS-7940, PI12/01511, PI11/00566, Spanish Rheumatology Society

II b Testosterone induces transgenerational impairments on the behavior of C. elegans. A model to explain androgen-dependent autistic traits

Authors: *María M. Gámez-Del-Estal, Israel Contreras, Rocío Prieto-Pérez, and Manuel Ruiz-Rubio.*
Group: GC20 *Genetic and Behavior Diseases*

Current research indicates that the causes of autism spectrum disorders (ASDs) are multifactorial and include both genetic and environmental factors. To date, several works have associated ASDs with mutations in genes that encode proteins involved in neuronal synapses; however other factors and the way they can interact with the development of the nervous system remain largely unknown. Some studies have established a direct relationship between risk for ASDs and the exposure of the fetus to high testosterone levels during the prenatal stage. Our research group has been pioneer using C. elegans in the study of genes involved in ASD. In this work, in order to explain possible mechanisms by which this androgenic hormone may interact with the nervous system, this nematode was used as an experimental model. We observed that testosterone was able to alter the behavioral pattern of the worm, including the gentle touch response and the pharyngeal pumping rate. This impairment of the behavior was abolished using specific RNAi against genes orthologous to the human androgen receptor gene. The effect of testosterone was eliminated in the nhr-69 (ok1926) deficient mutant, a putative ortholog of human AR gene, suggesting that this gene encodes a receptor able to interact with the hormone. On the other hand the testosterone effect remained in the gentle touch response during four generations in the absence of the hormone, indicating that some epigenetic mechanisms could be involved. Sodium butyrate, a histone deacetylase inhibitor, was able to abolish the effect of testosterone. In addition, the lasting effect of testosterone was eliminated after the dauer stage. These results suggest that testosterone may impair the nervous system function generating transgenerational epigenetic marks in the genome. This work may provide new paradigms for understanding biological mechanisms involved in ASDs traits.

II c Functional validation of SNP rs6105269 in Crohn's disease

Authors: P. Aguilar-Melero, G. Ferrín, J.M. Benítez-Cantero, E. Iglesias-Flores, I. Salgueiro, M.L. García, C. Linares, S. González-Rubio, M. de la Mata, V. García-Sánchez
Group: GC2 *Oxidative and Nitrosative Stress in Acute and Chronic Liver Disease*

Backgrounds & Aims: Nowadays, Crohn's Disease (CD) is one of the most prevalent inflammatory bowel diseases (IBD) in Europe (~70 cases/100,000 inhabitants). In spite of the numerous genetic studies that have identified several polymorphism of susceptibility to CD, their clinical translation is limited due to the lack of functional studies that associate these genes with the disease. The SNP rs6105269 (G to A) has been related to a higher risk for suffering CD in a genome-wide association studies. The aim of this study is to analyse the closest genes to this polymorphism, in order to associate their expressions with the pathology and to validate its feasible use as prognostic biomarkers. The genes near the SNP are MACROD2 and FLRT3. Methods: 95 patients with CD were enrolled. Plasma proteins codified by these genes were measured by ELISA in three groups of patients corresponding to the three different genotypes of the polymorphism rs6105269 (AA, GG and AG). The results show that protein MACROD2 is significantly downregulated in patients with the genotype AA (AA: 851 ng/ml, GG: 1372 ng/ml, AG: 907 ng/ml, p=0,04) and protein FLRT3 tends to be upregulated in these patients (AA: 13,9 ng/ml, GG: 10,2 ng/ml, AG: 11,2 ng/ml). Furthermore, 43% of AA patients has family history of IBD (GG 12 %, AG 9%, p=0,009), suggesting that the genetic background of AA patients is a significant risk factor for IBD. The AA genotype is link to stenotic and fistulizing patterns (AA 80% patients, GG 47% and AG 38%, p=0,02) and to a significant higher levels of the inflammatory marker, PCR (AA: 22,5 ng/ml, GG: 3,8 ng/ml, AG: 4,2 ng/ml, p=0,02). Conclusions: SNP rs6105269 downregulates MACROD2 expression in CD, promoting higher levels of inflammation and contributing to a more aggressive pattern (stenotic and fistulizing). Further studies are needed in order to discover MACROD2 functions.

II d Inflammation impairs regulation of FGF23 by phosphorus in renal failure

Authors: *Rodríguez-Ortiz ME, Díaz-Tocados JM, Muñoz-Castañeda JR, Herencia C, Martínez-Moreno JM, Montes de Oca A, Rodríguez M, Almadén Y*
Group: GC13 *Calcium Metabolism Vascular Calcification*

INTRODUCTION. Fibroblast growth factor 23 (FGF23) regulates phosphorus (P) and vitamin D levels after binding to an FGF receptor and its co-receptor Klotho in target tissues. Uremic patients show extremely high FGF23 levels. We aim to determine whether P restriction prevents the rise in FGF23 in a murine model of uremia. We also analyzed the contribution of inflammation to this increase.

METHODS. Normal male Wistar rats underwent 5/6 nephrectomy and fed diets containing 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2%P for either 3 or 15 days. In another experiment, uremic rats fed with diets containing 0.2 and 0.4%P were treated with LPS for 14 days to induce an inflammatory status. Blood and renal tissue were collected to evaluate mineral parameters and Klotho expression.

RESULTS. Plasmatic P increased progressively as dietary P increased at both 3 and 15 days. Animals feeding a 1.2%P diet had P levels significantly higher than controls at both 3 (11.225±3.697 mg/dl vs. 5.379±0.148 mg/dl in control rats, $P<0.05$) and 15 days (14.195±0.860 mg/dl vs. 6.830±0.071 mg/dl in control rats, $P<0.05$). FGF23 levels were significantly increased in all experimental groups at 3 days; however, only animals feeding diets containing 0.4%P or higher for 15 days had elevated FGF23. All groups treated with LPS showed higher FGF23 than their respective control groups (Sham-LPS 286.945±77.885 pg/ml vs. 92.545±12.680 in Sham, $P<0.05$; Nx0.2-LPS 112.737±31.858 pg/ml vs. 29.455±14.945 in Nx0.2, $P<0.05$; Nx0.4-LPS 472.751±95.325 pg/ml vs. 219.020±41.815 in Nx0.4, $P<0.05$). In addition, the increase in FGF23 in LPS-treated animals was accompanied by a decrease in the expression of renal Klotho.

CONCLUSION. Restriction of dietary phosphorus is associated with a decrease in FGF23 in rats after 15 days of uremia. In animals feeding a low P diet, administration of LPS stimulates FGF23 production, which is associated with a decrease in renal Klotho.

II e Transcriptional analysis reveals different immune regulation between scalp and skin psoriasis with potential therapeutic implications

Authors: *J Ruano, M Suárez-Fariñas, A Shemer, J.A. Marcusson, E Guttman-Yassky, A. Vélez-García Nieto, J Krueger.*
Group:

The scalp is frequently involved in psoriasis and it often constitutes the only manifestation of the disease. Scalp psoriasis shows a variable clinical spectrum and in many cases poses a great therapeutic challenge. We assessed in n=30 subjects the transcriptomic profile (by microarrays) of lesional and non-lesional psoriatic scalp tissue as compared with psoriatic skin. Clustering, principal component analysis, manually curated-gene set enrichment analysis, and co-differential expression gene set network analyses were performed. Our data suggest that the immune mechanisms that mediate scalp psoriasis are similar with those involved in psoriasis skin lesions. However, the magnitude of dysregulation [log2(FCH)], number of differentially expressed genes, and enrichment of the psoriatic genomic fingerprinting were higher for skin versus scalp lesions. Furthermore, the scalp transcriptome showed increased modulation of several gene sets, particularly those induced in keratinocytes by interferon-gamma, as compared with skin psoriasis samples that were mainly associated with a significant higher activation of genes involved in TNF-alpha/IL-17/IL-22-induced keratinocyte response genes. We also detected differences in expression of sets of genes involving negative regulation processes, psoriasis epigenetic regulation, epidermal differentiation, and dendritic cell or Th1/Th17/Th22-related T-cell function processes. These data help to understand the differences between skin and scalp psoriasis and may be valuable in selecting novel targets to specifically treat scalp psoriasis that still has a large unmet medical need.

II f Effects of calcitriol and paricalcitol on osteogenesis of bone marrow mesenchymal stem cells

Authors: *Herencia C, Díaz-Tocados JM, Martínez-Moreno JM, Montes de Oca A, Gómez-Luna MJ, Morales A, Rodríguez-Ortiz ME, Almadén Y, Rodríguez M, Muñoz-Castañeda JR*
Group: GC13 *Calcium Metabolism Vascular Calcification*

Hyperphosphatemia triggered in patients with chronic kidney disease (CKD), leads to the development of hypocalcemia, which is associated with the progression of secondary hyperparathyroidism (HPT). These alterations in mineral metabolism are closely related to vascular calcification (VC), a risk factor that contributes to increase the mortality rate in these patients. The VC is an active process similar to bone formation involving a variety of related mineral and bone metabolism proteins. Vitamin D (Calcitriol) and analogues (Paricalcitol) are used in the treatment of HPT from CKD patients. The bone plays a key role during phosphorus and vitamin D homeostasis. The effects of CTR or PC on osteogenesis of MSC are unknown.

The main objective of this study was to evaluate the effect of CTR and PC on osteogenesis from rat bone marrow mesenchymal stem cells (MSC). The effects of different concentrations of CTR and PC were evaluated during the process of differentiation of MSC into osteoblasts. Changes in mineralization (calcium content, alkaline phosphatase activity and alizarin-red staining), osteospecific genes expression (RT-PCR), intact FGF23 secreted (ELISA), proliferation (western-blot of PCNA and Cyclin D1) and finally nuclear β-catenin translocation (confocal microscopy) were studied.

The results showed that during osteogenesis high concentrations of CTR or PC decreased significantly mineralization and osteogenic markers such as Runx, Osterix and Osteocalcin. These changes were accompanied to low levels of FGF23 and a decrease of nuclear β-catenin translocation and cell proliferation. However, the administration of physiological doses of CTR or PC enhanced significantly mineralization, FGF23 levels, proliferation and nuclear translocation of β-catenin.

In conclusion, physiological concentrations of CTR (or its synthetic analog PC) contribute to enhance MSC osteogenesis however high concentrations of vitamin D or PC avoid it, decreasing FGF23 secretion and nuclear translocation of β-catenin promoting likely the generation of adynamic bone.

SESSION III

Cancer (Oncology and Oncohematology)

III a Hypoxia Response Induces the Formation of Polyploid Giant Cells and Cancer Stem Cells in Colon Cancer

Authors: *Lopez-Sánchez, L.M.; Jiménez, C.; Valverde, A.; Hernández, V.; Peñarando, J.; Martínez, Antonio; López-Pedrera Ch., Muñoz Castañeda JR; De la Haba JR; Aranda Aguilar E., Rodríguez Ariza, A.*
Group: GC6 *New Cancer Therapies*

The induction of polyploidy is considered the reproductive end of cells, but there is evidence that polyploid giant cancer cells (PGCCs) contribute to cell repopulation during tumor relapse. However, the role of these cells in the development, progression and response to therapy in colon cancer remains undefined. Therefore, the main objective of this study was to investigate the generation of PGCCs in colon cancer cells and identify mechanisms of formation.

Treatment of HCT-116 and Caco-2 colon cancer cells with the hypoxia mimic CoCl2 induced the formation of cells with larger cell and nuclear size (PGCCs), while the cells with normal morphology were selectively eliminated. Cytometric analysis showed that CoCl2 treatment induced G2 cell cycle arrest and the generation of a polyploid cell subpopulation with increased cellular DNA content. Polyploidy of hypoxia-induced PGCCs was confirmed by FISH analysis. Furthermore, CoCl2 treatment effectively induced the stabilization of HIF-1α, the differential expression of a truncated form of p53 (p47) and decreased levels of cyclin D1, indicating molecular mechanisms associated with cell cycle arrest at G2. Accordingly, pretreatment with pifithrin- α (PFT-α), which specifically blocks the transcriptional activity of p53, reduced the impact of CoCl2 on cell cycle and abrogated the formation of PGCCs. Generation of PGCCs also contributed to expansion of a cell subpopulation with cancer stem cells (CSCs) characteristics, as indicated by colonosphere formation assays, and induced higher chemoresistance to 5- fluorouracil and oxaliplatin.

In conclusion, the pharmacological induction of hypoxia in colon cancer cells causes the formation of PGCCs, the expansion of a cell subpopulation with CSC characteristics and chemoresistance. The molecular mechanisms involved, including the stabilization of HIF-1 α, the involvement of p53/p47 isoform and cell cycle arrest at G2, suggest novel targets to prevent tumor relapse and treatment failure in colon cancer.

Funded by MINECO-ISCIII: PI13/00553.

III b Lack of endogenous cortistatin but not somatostatin exacerbates carcinogen-induced mammary gland tumorigenesis in mice.

Authors: *Alicia Villa-Osaba, Manuel D. Gahete, Francisco Gracia-Navarro, Justo P. Castaño, Raúl M. Luque.*
Group: GC8 *Hormones and Cancer*

Somatostatin (SST) and cortistatin (CORT) are two structurally and functionally related peptides that share a family of receptors (sst1-5) to exert common biological actions, including the suppression of tumor cell proliferation. In fact, various ssts are abundantly expressed in normal mammary gland (MG) tissues and MG tumors, thereby offering a potential therapeutic target. However, attempts to apply SST analogs in breast cancer have been unsuccessful hitherto, and the specific role of SST and/or CORT in MG tumorigenesis remains uncertain. Here, we studied the influence of endogenous SST and CORT on carcinogen-induced MG tumorigenesis, by treating female SST- and CORT-knockout (KO) mice and their respective littermate controls (n=9-16/group; FVN/B background; 22-wk-old) with 7,12-dimethyl-benza-anthracene (DMBA; 0.5mg/10g BW; once/wk for 3 wk) and MG tumorigenesis was followed for 24 wk. Surprisingly, lack of SST did not impact DMBA-induced MG tumor incidence [1/13 (8%) and 2/16 (13%) mice developed MG tumors in SST-KOs and controls, respectively]. However, lack of endogenous CORT aggravated DMBA-induced MG tumorigenesis, as 8/14 (57%) CORT-KOs developed MG tumors compared to controls [1/9 (11%)]. Additionally, tumor latency was reduced while tumor multiplicity increased in CORT-KOs compared with controls. These results are supported by MG whole mount analyses, which revealed higher incidence of neoplastic or preneoplastic lesions in CORT-KOs compared with controls [6/14 (43%) and 3/9 (33%), respectively]. Of note, lack of endogenous SST or CORT did not significantly alter local expression of ssts in MG, neither the expression of the main components of the GH/IGF-I system, which play relevant roles in MG tumorigenesis and are regulated by SST/CORT in other tissues. Altogether, our data demonstrate that endogenous SST and CORT distinctly contribute to the control of DMBA-induced MG tumorigenesis, and thereby suggest that CORT, rather than SST, might act as a key inhibitor factor of MG tumorigenesis in mice.

III c The fungal metabolite galiellalactone blocks cell cycle progression in prostate cancer cells through STAT3 and NF-kB independent pathways

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Group: GC4 *Inflammation and Cancer*

Background: Prostate cancer is the second most common cancer in men worldwide. Initially, prostate cancer cells respond to androgen deprivation therapy but within 12-18 months many patients
develop castration-resistant prostate cancer (CRPC) with a need for second-line therapy still unavailable. The transcription factors NF-kB and STAT3 play a key role in CRPC and therefore are validated targets for the development of new therapies. We have recently shown that the small molecule Galiellalactone (GL) is a dual NF-kB and STAT3 inhibitor (1,2). Since NF-kB and STAT3 regulate some cell cycle checkpoints we have investigated the effect GL on cell cycle in prostate cancer cells and the mechanism of action underlying this activity.
Methods: The prostate cancer cell lines DU145, LNCaP, LNCaP-IL-6, PC-3 and human prostate epithelial RWPE-1 cells were treated with GL and cell cycle arrest analyzed by flow cytometry. The effects of GL on key proteins that regulate cell cycle checkpoints were identified by western blots. The activity of NF-kB, STAT3, STAT1 and STAT5 was investigated by transient transfection using specific luciferase reporter genes. Actin aggregation was studied by fluorescence microscopy. IL-6-induced autophagy was analyzed by flow cytometry. Finally the role of STAT3 and NF-kB on cell cycle was investigated using DU145-KO-STAT3 and DU145-KO-NF-kB cell lines generated by infection with lentivirus containing specific shRNAs.
Results: GL induced cell cycle arrest at the G2/M phase in androgen insensitive DU145 and PC3 cells but not in androgen-sensitive LNCaP and epithelial RWPE-1 cells. GL inhibited NF-kB, STAT3 transcriptional activities in DU145 cells and also induced cell cycle arrest in DU145-KO-STAT3 and DU145-KO-NF-kB cells. Moreover GL induced actin aggregation and selectively modulated the expression of cell cycle related proteins (cdc25C, Cyclin A, Cyclin E, myt-1, wee-1). IL-6-induced autophagy in LNCaP was not prevented by GL treatment.
Conclusion: GL induces actin aggregation, G2/M cell cycle arrest and apoptosis in androgen independent prostate cancer cells. The effect of GL on cell cycle arrest is cell-type dependent and does not require the participation of NF-kB and STAT3. In conclusion GL is a multi-target compound that could serve as a lead compound for the development novel CRPC treatments.

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III d In vivo effect of 5-azacitidine treatment in regulatory t cells in patients with aml / mds

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Group: GC16 *Cell Biology in Haematology. Hypercoagulability*

Introduction: 5-azacytidine (5-Aza) is a hypomethylating agent that improves outcome in patients with high risk Myelodysplastic Syndrome (HR-MDS) and in Acute Myeloblastic Leukemia (AML) with £30% blasts. It has been demonstrated that 5-Aza increases regulatory T cells CD4 + CD25 + CD127lowFoxP3 + (Treg) in vitro and in murine models. The objective of this study is to analyze the in vivo effect of 5-Aza treatment in Treg in patients with LAM / SMD, the kinetics and correlation with clinical and biological data..

Patients and Methods: We included 19 5-Aza treated patients,. 52.7% (n = 10) were LAM and 47.9% HR-MDS. 5-AZA dose was 75-100mg/m2/day 7days monthly . Treatment responses were defined according International Working Group criteria 2006.

Total CD4+ and Treg population were quantified in PBMC samples obtained: 1) Pre-treatment, 2) Early (<3 m), 3) Intermediate (3-6 m) and 4) Late (> 6 m). We analyzed 96 samples. At least 1x104 CD4total events were acquired in a FACSCanto II, labelled with the following MnAbs: CD4-FITC (RPA-T4), CD25-APC (2A3), CD127-PE (hIL-7R-M21), Foxp3-PE (259D/C7) and IgG1-PE (X40) as isotype control.

Results: With A median follow-up of 5 months (1-19) and 6 cycles administered (1-19), we observed 36.8% (n = 8) of Overall Response (CR / PR) and 31.6% (n = 6) hematologic improvement with transfusion independence (IT) 26.3% (n = 5). Total CD4 and Treg in patients after 5-aza were significantly increased compared to basal pre-treatment levels and compared to healthy subjects (P= 0.001 and P= 0.004, respectively). Maximum peak was reached at 3-6 months after 5-Aza treatment. We found a higher percentage of Treg in LAM compared to SMD at 3-6 m point (13.78 vs. 6.13%, p = 0.03) and› 6m point (18.09 vs. 6.87%, P= 0.01). Treg percentages were not statistically different comparing age, sex, MO % blasts, karyotype, 5-Aza dose schedule or infections Patients who achieved OR had significantly lower percentage of total CD4 and Treg percentages compared to those without response (3.17% vs.15.89% and 11.3% vs. 38.48%, P = 0.05). We also found lowere total CD4 (19.38% vs. 30.8%) and Treg (6.68% vs. 10.68%) in patients achieving peripheral response vs no response (P = 0.05) at 3-6 m, and patients with IT (% Treg: 6.68 vs. 10.68%, P = 0.05) at 3-6 m.

Conclusions: 5-Aza produces in vivo an augment in the percentage of Treg cells gradually reaching a maximum peak at 3-6 months. Patients with LAM have more Treg percentage compared to MDS patients. Patients who achieve response (OR /HI) with 5-Aza had a significantly lower percentage of Treg in patients compared to those without response.

III e Modern surgical liver resections offer better results than transarterial chemoembolization in cirrhotic patients with b-bclc stage hcc

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BACKGROUND. According to the Barcelona Clinic Liver Classification (BCLC), transarterial chemoembolization (TACE) is accepted as the best treatment for BCLC B-stage hepatocellular carcinoma (HCC) for cirrotic patients, being palliative and offering a reduced survival with a median of 19-20 months.
OBJECTIVES. Our aim was to analyze the impact of liver resection (LR) versus TACE on this patients.
PATIENTS AND METHODS. Retrospective analysis of patients with B-BCLC HCC treated with both LR or TACE between 2006-2012 in our tertiary hospital. Data exposure: Mean (SD) and %. Overall survival, free-of-disease and recurrence (log-rank-test and multivariate-Cox).
RESULTS. 80 patients diagnosed and treated with BCLC B-stage HCC in our centre were included (45 TACE and 35 LR). The mean age was 66 (10) years. The number of nodules was 1.84 (1.13) being higher in the TACE group [2.16(1.15)] compared with the LR group[1.43(0.98)]. The mean survival period was 774,54 days (median follow up of 615 days), with a global mortality of TACE vs. LR of 55,5% vs. 31,4%, respectively. Survival at 1-, 2- and 3- years of TACE vs. LR group was 71.1%, 55.6% and 44.4% vs. 80%, 71.4% and 68.6%, respectively (p=0.011). The overall recurrence rate in TACE group vs. LR was 44.4% vs. 34%, respectively. The mean time to recurrence was 484.24 (428.57) days, with a mean free-of-disease period for TACE and LR of 360,58 (378.46) vs. 643.23 (441.76) days, respectively ($p<0.05$).
The multivariant analysis showed that tumor size(HR=1.146[1.029-1.276]) and the use of TACE(HR=4.048[1.694-9.673]) were predicting factors of overall survival with an end-point of 36 months. Only TACE(2.091[0.995-4.395]) reached a value near the statistical significance (p=0.052) when analysing the free-of-disease survival after 36 months.
In subgroup analysis for the LR group, the univariant analysis showed a specially good prognosis in 36 months (p=0.04) in cases of good/moderate tumor differentiation and abscense of vascular invasion (92.3%) compared with the rest of the groups. The multivariant analysis showed that good/moderate differentiation without vascular invasion represented a good prognostic factor of overall survival(HR=8.774 [1.116-68.986]) and recurrence(HR=10.733 [1.35-85.03]) in this subgroup.
CONCLUSIONS. Management of B-BCLC HCC patients should be more complex. Modern surgical resection offers excellent survival benefit with optimal security, especially in patients with good/moderate differentiation with no vascular invasion.

SESSION IV

Nutrition, Endocrine and metabolic diseases

IV a SEPT11: a novel adipocyte marker related to obesity and insulin resistance

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Group: GC11 *Metabolism and adipocyte differentiation. Metabolic Syndrome*

Introduction: In obesity, adipocytes undergo rearrangements in the expression and distribution of numerous proteins, including cytoskeletal components. In a proteomic analysis of subcutaneous adipose tissue (SAT) from morbidly obese patients SEPT11 was identified based on its differential regulation in normoglycemia (NG), insulin resistance (IR), and weight loss. SEPT11 belongs to a protein family considered as the fourth component of the cytoskeleton. Herein, we sought to: (i) characterize SEPT11 in human AT in relation to obesity and type 2 diabetes (T2D), and (ii) determine the cellular and molecular features of SEPT11 and its interaction with other cytoskeletal elements and associated structures (caveolae).
Methods: SEPT11 and caveolin-1 mRNA and protein content were measured in SAT and omental AT (OAT) from lean (n=10), and obese patients [NG (n=24) and T2D (n=30)], as well as in omental adipocytes isolated from obese patients and treated with insulin, leptin, isoproterenol, TNFα or LPS. SEPT11 intracellular distribution was studied in human and 3T3-L1 adipocytes by confocal microscopy and subcellular fractionation. Finally, Yeast-Two Hybrid (Y2H) was used to identify SEPT11 interacting proteins.
Results: SEPT11 expression was predominant in adipocytes, and increased in relation to obesity and T2D. In SAT, SEPT11 positively correlated with SEPT2, SEPT9 and Cav-1, but only with SEPT9 and Cav-1 in OAT. In vitro, insulin increased, whereas isoproterenol, TNFα and LPS decreased SEPT11 expression. In relation to its intracellular localization, SEPT11 formed ring-like structures reminiscent of caveolae or distributed as bundles resembling actin filaments. Finally, our Y2H assay enabled identification of several interacting proteins, including actin polimerization regulators (ARHGAP21) and lipid transporters (FABP5).
Conclusion: Our data suggests that SEPT11 represents a novel component of the cytoskeleton in adipocytes that, through its relation with actin filaments and caveolae, may play a role in the regulation of adipocyte physiology in obesity and IR.

IV b Expression of Mkrn3 and mir-30b in the hypothalamus during postnatal maturation and in preclinical models of altered puberty: A novel regulatory system in the control of puberty onset?

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MKRN3, the gene encoding the makorin RING-finger protein 3, has been very recently suggested to play a key role in the control of puberty onset, since (i) genetic inactivation of MKRN3 has been described in patients with central precocious puberty and (ii) hypothalamic Mkrn3 expression has been shown to decline before puberty in mice. Altogether, these data would suggest a repressive role of MKRN3 in pubertal timing; yet, the actual roles of MKRN3 in the pathophysiological regulation of puberty onset remain largely unexplored.

To address this issue, we report here the expression profiles of Mkrn3 mRNA in the hypothalamus of female rats during postnatal maturation and in selected models of altered puberty. In parallel, hypothalamic expression analyses of the putative microRNA (miRNA) regulator of Mkrn3, mir-30b, as predicted by bioinformatics algorithms, are also presented. Of note, miRNA pathways have been recently proposed as novel central regulators of puberty; miRNAs being mainly repressors of protein expression either by promoting mRNA degradation or preventing translation of target mRNAs, with whom they interact at specific seed regions at the 3'-UTR.

Consistent with previous studies in mice, hypothalamic Mkrn3 mRNA in female rats displayed very high expression during the neonatal period, markedly decreased during the infantile-to-juvenile transition and reached minimal levels before puberty; this profile is compatible with the hypothesis that a decline in Mkrn3 expression is permissive for the central activation of puberty. Notably, the expression profile of mir-30b in the arcuate nucleus (ARC) was opposite to that described for Mkrn3, with minimal neonatal levels and progressive increases along postnatal maturation. Such an inverse mir-30b/Mkrn3 ratio is in keeping with a role of this miRNA in the negative control of Mkrn3.

In addition, perturbations in pubertal development induced by neonatal exposure to high doses of sex-steroid acting compounds or early postnatal underfeeding altered Mkrn3 expression patterns. Thus, neonatal treatment with a synthetic estrogen, estradiol benzoate, in a protocol known to disturb brain sex differentiation and puberty, significantly enhanced Mkrn3 expression in the hypothalamus of pubertal rats. Likewise, in female rats subjected to postnatal sub-nutrition, which display delayed puberty, the expression levels of Mkrn3 failed to decline as in normal postnatal development (i.e., remained at a high levels during the infantile-to-juvenile transition). Expression analyses are in progress to evaluate changes in the hypothalamic levels of mir-30b in the above preclinical models of perturbed puberty.

In sum, our data support recent findings on the putative role of Mkrn3 in the central control of puberty, with a presumable suppressor function (on as yet unknown elements of the reproductive brain), which is lifted during (pre)-pubertal maturation, as suggested by the dramatic decrease of hypothalamic Mkrn3 mRNA levels preceding puberty. Our study is the first to document an inverse relationship in the ARC levels of Mkrn3 and its predicted negative regulator, mir-30b, and to demonstrate substantial changes in the hypothalamic expression of Mkrn3 in preclinical models of altered puberty. Altogether, these findings suggest the potential involvement of developmental changes in Mkrn3/mir-30b at the hypothalamus in the central mechanisms permitting/leading to puberty onset, and may pave the way for a better understanding of the basis for altered puberty in conditions of metabolic stress and/or hormonal disruption.

IV c Levels of fitness and physical activity influence changes on response of plasma adipokines at prepubertal stage

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Group: GE2 *Child Metabolism*

Introduction. Physical activity and cardiorespiratory fitness could be protective against low-grade inflammation. Therefore, the aim of this study was comparing plasma levels of a relevant group of adipokines and inflammation biomarkers in prepubertal healthy children with different levels of fitness and physical activity.
Methods. 132 healthy prepubertal children aged 7-12 years were recruited from local schools. Children were divided into two groups in relation to fitness –low fitness (LF) and high fitness (HF)– according to the 20-meter shuttle run test results. The same sample was also divided in relation to the exercise practice evaluated through an after-school programme and questionnaires: sedentary group (SG) and physical activity group (PAG). Anthropometric parameters and the following plasma adipokines were measured: leptin, resistin, adiponectin, alpha tumor necrosis factor, hepatic growth factor, interleukin (IL) 6 and 8, macrophage chemoattractant type-1 (MCP1), nerve growth factor, and plasminogen activator inhibitor-1.
Results. fter adjustment for BMI, age and sex, the LF group showed higher leptin levels and lower IL-6 levels as compared to the HF group. In relation to PA, adiponectin and MCP1 levels were higher in the SG as compared to the PAG but after adjustments, only MCP1 remained significant. When boys and girls were compared, no differences were found.
Conclusions. Sedentary and children with a low level of fitness showed altered inflammatory cytokine levels, as compared to children with high fitness or actives, already in prepubertal age and regardless of BMI, age and sex. The protection provided by adequate levels of fitness and physical activity against inflammation damage in childhood should be further studied to prevent the development of diseases in adulthood.

IV d Beneficial Effect of CETP Gene Polymorphism rs3764261 in Combination with a Mediterranean Diet on Lipid Metabolism in the Patients with Metabolic Syndrome

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Group: GE9 *Nutrigenomics. Metabolic Syndrome*

Introduction: Cholesteryl ester transfer protein (CETP) gene has been implicated in high-density lipoprotein (HDL-C) metabolism. However, little is known about the impact of this gene on metabolic syndrome (MetS) patients and its interaction with diet.
Objective: To evaluate whether the chronic consumption of a Mediterranean diet enriched in olive oil, compared with a Low fat diet, interacts with the rs3764261 SNP at CETP locus in order to modify lipid metabolism among MetS patients from the CORDIOPREV clinical trial.
Methods: Plasma lipid concentrations and rs3764261 genotypes were determined in 424 MetS subjects participating in the CORDIOPREV clinical trial (NCT00924937). Gene-diet interactions were analyzed after a year of dietary intervention (Mediterranean diet (35% fat, 22% MUFA) vs low fat diet (28% fat, 12% MUFA)).
Results: We found significant gene-diet interactions between rs3764261 SNP and the dietary pattern for HDL-C (P=0.006) and triglyceride concentrations (P=0.040). Specifically, after 12 months of Mediterranean diet intervention, subjects who were carriers of the minor A allele (AA+AC) displayed higher plasma HDL-C concentrations (P=0.021) and lower triglycerides (P=0.020) compared with homozygous for the major allele (CC). In contrast, in the low fat intervention group no significant differences were found between CETP genotypes after 12 months of dietary treatment.
Conclusions: Our data support the notion that a chronic consumption of a Mediterranean diet may play a contributing role in triggering lipid metabolism by interacting with the rs3764261 SNP at CETP gene locus in MetS patients. Due to the complex nature of gene-environment interactions, dietary adjustment in subjects with the MetS may require a personalised approach.

IV d Genetic targets which could determine the shortening of telomeres in obese people

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Introduction: Telomeres are chromosomal structures whose function is the maintenance and integrity of the genome. Telomere length has been defined as a parameter of cellular aging. This parameter can be regulated by environmental, genetic and lifestyle factors.
The aim of this work was to study the relationship between some anthropometric, and biochemical parameters, and genetic aspects with the telomere length in obese people.
Materials and Methods: 926 patients between 20 and 75 years were selected with cardiovascular disease and metabolic syndrome (CordioPrev). Biochemical and anthropometric measurements were made and the telomeres length (RTL) was measured by real time PCR. The statistical relationship between RTL and anthropometric and biochemical parameters were analysed. Also the correlation between RTL and genotype of patients for single polymorphisms (SNPs) located in the TNF - α , AdipoR1 and LPL genes was studied.
Results: We observed a less telomere length in obese people with waist perimeter higher than 108.6 cm (p = 0.044), weight higher than of 88 kg (p = 0.030), and body mass index (BMI) higher than 30 kg/m2 (obesity type I- II; p = 0.039, and obesity type III; p = 0.025). A less telomere length was also observed in function of heterozygous vs homozygous genotypes, for SNP's located in the genes: TNF- α (p = 0.048) , AdipoR1 (p = 0.010) and LPL (p = 0.010), genes directly related to obesity.
Conclusion: In patients with cardiovascular disease and metabolic syndrome, obesity is a risk factor directly linked to reduced telomere length. In addition, there is a genetic predisposition to obesity that in turn could determine the length of telomeres.

POSTER SESSION

1 The mTOR inhibitor everolimus is safe within the first month after liver transplantation

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Group: GC2 *Oxidative and Nitrosative Stress in Acute and Chronic Liver Disease/* **GC18** *Translational Research in Surgery of Solid Organ Transplantation*

BACKGROUND: The mTOR inhibitor sirolimus should not be started within the first month after liver transplantation (LT) because of an increased risk of hepatic artery thrombosis (HAT) found in the registry trial. The evidence regarding everolimus is lacking but the manufacturer's recommendations transposed the same warning. We aimed to evaluate the safety of everolimus started within the first month after LT.
METHODS: We retrospectively evaluated 187 consecutive LT patients at the Reina Sofía University Hospital (2009-2013). Patients starting everolimus within the first month after LT were compared with those starting everolimus thereafter or not receiving this drug. Median follow up was 21 months (IQR 7-36). Kaplan-Meir curves and Log-rank test were used to evaluate outcome.
RESULTS: Everolimus was started within the first month after LT in 33 patients (17.6%), with a median interval from LT of 12 days (IQR 8.5-20.5). Twenty-five patients (13.4%) started the drug thereafter (median day 90; IQR=37-365), and 129 patients (69%) did not receive everolimus. The incidence of HAT was reduced in patients early treated with everolimus when compared with the remaining cohort (0% vs 10.6%; p=0.036). No wound healing complications were detected in the early everolimus group. There were similar rates of incisional hernia (p=0.31), infections (p=0.15), renal impairment (0.43), and histologically proven acute cellular rejection (p=0.32) between groups. Hyperlipidemia rates were increased in the group early treated with everolimus (42.6% vs 3.6% at 3 years; p=0.018). There were neither differences in terms of graft loss (12.6% with early everolimus vs 21.3% with late or no everolimus at 3 years; p=0.25), nor regarding overall mortality (34.8% with early everolimus vs 29.1% with late or no everolimus at 3 years; p=0.88).
CONCLUSION: Everolimus proved to be safe within the first month after LT, and registered reduced HAT. Randomized controlled trials are warranted to confirm our findings.

2 Effects of exogenous lipid sources on mitochondrial physiology and ultraestructure in an in vitro model.

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Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Many scientific research groups have focused their interest on the processes that are involved in aging as well as in their possible manipulation in order to stop or retard it. Although many possible mechanisms have been identified and several theories to explain aging have been formulated, the unsaturation level of the membrane phospholipids (Membrane Theory of Aging) (Hulbert, 2003) and the endogenous generation of reactive oxygen species (ROS) (Mitochondrial/Free Radicals Theory of Aging) (Harman, 1956) are two of the most widely accepted factors that affect aging.

The unsaturation degree of cellular membranes is inversely proportional to longevity, although unsaturated fatty acids are also crucial to maintain the fluidity of the lipid membranes. Fatty acid content of the diet can modify the composition of membrane phospholipids (Ochoa-Herrera et.al, 2001), and this can affect the function of the electron transport chain (ETC) located in the inner mitochondrial membrane (Aoun et.al, 2012), which is not only the most important cell energetic machinery for ATP generation, but also one of the main sources of ROS in cells.

The goal of this study was to determine how the mitochondrial ultraestructure and physiology could be affected by supplementation with exogenous lipids using an in vitro model. Cultures of a mouse hepatocarcinoma cell line (Hepa 1.6) were supplemented with two different lipid emulsions: Lipofundin, based on ω-6 fatty acids (thus mimicking a diet enriched in soybean oil) or Lipoplus which also contains ω-3 fatty acids (thus mimicking a diet containing fish oil). After 48 hours of treatment (7μg/ml of emulsion), different mitochondrial and oxidative stress parameters were measured: Intracellular levels of superoxide (Het) and peroxides (DCFH-DA), mitochondrial superoxide (MitoSOX) and mitochondrial membrane potential ($\Delta\Psi$) (JC-1) were assessed by flow cytometry; activities of ETC complexes were assessed by enzymatic assays; finally, putative ultrastructural modifications were studied by planimetric and stereological measurements carried out from electron microscope micrographs.

Our results indicate that exogenous fat targets mitochondrial morphology and physiology in a manner that is dependent on fatty acid composition. Both Lipofundin and Lipoplus produce a significant increase in mitochondrial superoxide levels and in the number of mitochondria per cell, while $\Delta\Psi$ and mitochondrial volume density (Vv) are only significantly augmented with Lipofundin.

3 Non-3 induces oxidative stress and apoptosis in HEPG2 cell line in a process mediated by FDXR and cathepsin-D

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Group: GC2 *Oxidative and Nitrosative Stress in Acute and Chronic Liver Disease*

Introduction: Stable overexpression of endothelial nitric oxide synthase (eNOS/NOS-3) in the human hepatocarcinoma cell line HepG2 (HepG2/NOS-3) is associated with increases in respiratory activity, ATP production, and cell death. In addition, NOS-3 overexpression induces p53 and CD95, making the cells more susceptible to anti-Fas-induced cell death.
Objective: To study the protein expression profile of HepG2/NOS-3 in basal conditions as well as in anti-Fas-induced cell death, in order to identify those proteins differentially expressed related with NOS-3 overexpression and the processes described above.
Material and Methods: Cell death was induced by anti-Fas agonist (0.5 mg/ml; MBL) for 2 hours, and was evaluated by caspase-9/-3 activity. Oxidative stress and mitochondrial membrane potential were determined by spectrophotometry. Proteomic analysis was performed by 2D-PAGE coupled with MALDI/TOF-TOF MS. 2D-PAGE results were statistically analyzed, using PDQuest8.0.1 software, and verified by western-blot.
Results: NOS-3 overexpression was associated to oxidative stress, mitochondrial membrane potential and caspase-9/-3 activity increases. The proteomic study identified proteins mainly related to the antioxidant system, as well as metabolism or cellular architecture. In addition, we identified the proteins NADPH:adrenodoxin oxidoreductase mitochondrial (FDXR), which contributes to p53-mediated apoptosis through free radical generation, and Cathepsin-D, a protease that participates in apoptosis induced by nitric oxide.
Conclusion: NOS-3 overexpression promotes oxidative stress and apoptosis in HepG2 cell line, in a process characterized by the increase of the enzymes FDXR and Cathepsin-D. These results agree with those others that demonstrate that NOS-3 overexpression can be a potentially useful strategy for the experimental hepatocarcinogenesis treatment.

4 The role of machine learning classifiers to predict early recurrence of hepatocellular carcinoma after liver transplantation

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BACKGROUND AND AIMS: Despite of a careful selection of patients with hepatocellular carcinoma (HCC) for liver transplantation (LT), tumor recurrence rates are 15%-20%. We explored the role of machine learning classifiers to identify patients at increased risk of HCC recurrence within 3 years after LT.
METHODS: 185 consecutive patients with HCC receiving a LT between 2000-2010 at 2 centres were included: Reina Sofía University Hospital (n=91; training cohort) and Royal Free Hospital (n=94; validation cohort). Exclusion criteria were: combined transplantation and death within 3 years after LT not caused by HCC recurrence. Machine learning classifiers, which are multivariate models with a hybrid structure (a nonlinear part from an artificial neural network, and a linear part from a logistic regression model) were used to identify patients at increased risk of early HCC recurrence after LT.
RESULTS: HCC recurrence rates at 3 years were 16.2% (30/185). Patients experiencing HCC recurrence had increased diameter of the main nodule (4,3±2,8 cm vs 2,6±1,2 cm; p <0,001), and increased total tumour size defined as sum of diameters of each nodule (5,3±3,2 cm vs 3,3±1,7 cm; p<0,001). The predictive model included the following variables with their associated normalized coefficients (NC): Gender (NC=-0,114), time in waiting list (NC=0,096), HCV cirrhosis (NC=-1,085), diameter of the biggest nodule (NC=-3,832) and total size of HCC (NC=-1,982), the last 2 evaluated by imaging techniques. The AUROC in the whole cohort was 0.8012. The accuracy was 78.02% in the training cohort and 89.36% in the validation cohort. A threshold >0.35 selected patients at very high risk of HCC recurrence (41.1% in the training cohort and 35.7% in the validation cohort)''.
CONCLUSION: Machine learning classifiers allow to select patients at increased risk of early HCC recurrence after LT. For such patients alternative approaches should be considered.

5 Effects of age and CMV infection in the cytotoxic function of NK cells

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NK cells are an important component of the innate immune response against virus infected cells and tumor cells. Age associated changes in NK cell phenotype have been previously reported that can be responsible of functional NK cell deficiency. This work analyze the effect CMV seropositivity and aging on basal expression of cytotoxic granules (granzymes A / B and perforine) and the expression of cell surface receptors (CD94/NKG2 heterodimers and CD57) in these NK cell subsets. Our results show that CMV seropositivity in healthy young or elderly individuals is associated to the expression of CD94/NKG2C dimers and high expression of CD57 on the CD56dimCD16+ NK cell subset. CD56-CD16+NK cells, which are expanded in the elderly, show a decreased expression of granzymes A and B and an increased expression of CD94/NKG2C and CD57 in CMV seropositive young donors when compared with CMV seronegative young individuals. These results indicate that CMV and age have a different effect on NK cell phenotype and emphasize the relevance of including the determination of CMV serostatus in those studies addressed to analyze the immune response in the elderly.

6 A model of cholestasis in vivo promotes AP-1 and SP1 activation and Nos-3 inhibition, and reproduces the observations in HepG2 cell line

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Background: Retention of bile acids during cholestasis contributes to the development of liver damage. The reduction of endothelial nitric oxide synthase (eNOS/NOS-3) expression acts as a key factor in progression of liver injury in vivo. GCDCA-induced cell death is associated to NOS-3 expression/activity decrease by oxidative stress. This fact is related to JNK and ERK1/2 phosphorylation, and Nos-3 promoter binding increase of cJun, cFos and SP1.
Aim: To determine if hepatocellular damage in a model of cholestasis in vivo is associated to oxidative stress and proceeds through cJun, cFos and/or SP1 activation, and Nos-3 expression inhibition.
Methods: Male Wistar rats were randomly assigned to each group: 1) Control group with sham operation (SO, n=13); 2) Bile duct ligation group (BDL, n=13). Animals were sacrificed 7 days after surgery. Liver damage was determined by serum quantification of GGT, AST, ALT, and total/direct bilirubin, and by histological examination. Oxidative stress was evaluated by RT- qPCR, studying the expression of Cu/Zn-SOD, Mn-SOD, GPX4, GPX1, and catalase. Cell death was assessed by caspase-3 activity in liver lysates. Nos-3 and transcription factors (TFs) expression was determined by RT-qPCR and western-blot.
Results: BDL increased caspase-3 activity and serum levels of liver function markers. This was associated to hepatic fibrosis, bile ductules proliferation, antioxidant enzymes expression decrease, TFs activation increase, and Nos-3 expression inhibition.
Conclusions: The animal model of cholestasis reproduces previous observations in HepG2, where the oxidative stress generated by GCDCA addition causes AP-1 and SP1 activation and their binding to the Nos-3 promoter, inhibiting the NOS-3 expression.

7 Long-term allografts survival after kidney transplantation

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Introduction and objectives. Technical and medical advances over the last few years have produced an important increase in the functionality of renal allograft. The aim of this study is to identify which are the factors associated with allograft survival after 15 years since the transplantation in our series.
Materials and methods. A retrospective study of the kidney transplants carried out in Reina Sofia Hospital of Cordoba between February 1979 and December 1997 with follow up until June 2012. A subanalysis of the series is undertaken, and a Kaplan-Meier analysis and Cox proportional hazard model regression are used to achieve the main objective of the study.
Results: 487 renal allografts with a mean follow up of 114 months are studied of which 37% (N=180) survived for more than 15 years. Of the 180 patients, the main cause of graft failure is chronic allograft nephropathy in 29 (66%) and patient death in 13 patients (29.5%). Multivariate analysis identified the number of HLA mismatches (HR 1.25: 95% CI; 1.01-1.56), panel reactive antibodies (HR 2.68: CI; 1.28-5.26), and delayed graft function (HR 11.25: CI; 1.33-95.28) as significantly associated with graft loss after 15 years.
Conclusion: The high immunological risk of the patients was associated with graft loss in an independent way. Delayed graft function was the most important factor in the speed of graft failure beyond 15 years.

8 Temporal rhythmic patterns in falls in the elderly: insights for prevention

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Group: GA2 *Comprehensive nursing care. Multi-disciplinary perspectives*

Falls represent common and serious problems among older people and their families worldwide, and social and economic burden as well. One-third of people aged 65 and older fall at least once a year, and the risk increases with age (Chang and Ganz 2007).

At today, when falls occur is not often reported. It is known that acute diseases do not occur randomly over time, but exhibit temporal variations. In particular, an Italian group has pioneered the identification of highest risk timeframes for occurrence of many acute diseases: myocardial infarction, stroke,... which have higher frequency during morning hours (Gallerani et al. 1992, Casetta et al. 2002, Mehta et al. 2002, Manfredini et al. 2004, Manfredini et al. 2005).

Regarding to fall, evidence show different highest risk timeframes of falls according to the setting in which the study takes place. In long-term care institutions, several studies (Bouwen, 2008; Pelfolk, 2009) have studied the temporal specificity of falls in elderly with Alzheimer's disease observing a peak incidence of falls at 6 pm. In hospital setting, falls occur not only at night as commonly believed, but also during late-morning. In fact, Manfredini et al. 2011 employed a validated chronobiological method of analysis to document the identification of rhythmic patterns and they found a biphasic pattern characterized by peaks during the morning and late afternoon, with a main peak at 10.28 am. Also, winter-autumn seem to be the higher risk season (IRR=1.91, CI= 1.32 to 2.75), whereas no day-of-week periodicity has been reported (McMahon et al. 2012).

These funding highlight the importance of cronobiological analysis to identify high risk timeframes. Moreover, adding precise indication of time of falls for each setting could provide useful additional information to know the risk factors which may contribute to falls. And therefore, precise indication of time could help to find possible risk factors on each vulnerable timeframe for each setting, and appropriate specific countermeasures could be applied.

9 Elder abuse situation in South America and the Iberian Peninsula

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Objective: To dimension and compare the situation of elder people (EP) in the families and communities in South America and Iberian Peninsula.
Methods: a bibliographical search was performed in different databases: Health&Medical, PubMed and Google Scholar using as search strategy keywords: ''Elder abuse'' OR ''Violence AND Elderly''. In the case of Bolivia as there was no previous studies, a descriptive study was performed with a sample size of 50 60-year-old people coming from different Healthcare areas of Santa Cruz de la Sierra, It was used as dependent variable the Elder Abuse Suspicion Index (Perez-Rojo et al., 2010) (case of possible abuse if one or more affirmative responses of item 2 to 6).
Results:
- In Bolivia, it was identified an abuse rate of 46% of the EP participating in this study.
- In Brazil, 20.8% of respondents reported experiencing at least one type of violence in their home environment (Duke, 2012).
- In Chile, one in five EP has been the victim of some type of abuse, increasing allegations of abuse by 132% between 2010 and 2011 (Terra, 2013).
- In Peru, 50% of EP experience some form of abuse from their families (Andean Daily, 2013).
- In Portugal, 39.4% of EP are victims of domestic violence (Journal News, 2011).
- In Spain, the prevalence of abuse towards EP over 60 in the family, is between 3% and 12% (Moya, 2005).
Discussion: data obtained show the problem of elder abuse (EA) in the world, being present in every setting studied with alarming data. This demonstrates the urgent need for research on the prevalence and health consequences of EA, both in developing and underdeveloped countries.
Conclusions: the prevalence of abuse suffered by EP is an alarming magnitude in all contexts studied. The immediate families perpetrate most of the abuse to EP and the most common form of abuse is psychological.

11 Essential role of α-MSH signaling in mediating the permissive effects of leptin on puberty onset and its interplay with kisspeptin pathways

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POMC neurons in the hypothalamic arcuate nucleus (ARC) play an essential role in energy homeostasis, partially via the release of α-MSH that acts through melanocortin 3 and 4 receptors (MC3R and MC4R) to suppress food intake and mediate the anorectic effects of leptin. α-MSH pathways seem to be involved also in the central control of reproduction and bidirectional interplay with kisspeptins, ligands of Gpr54 and indispensable elements of the reproductive brain, has been suggested. Yet, the role of α-MSH in the regulation of puberty onset remains virtually unexplored. In our preliminary set of studies, we documented that the a-MSH agonist, MT-II, injected centrally elicited robust LH responses, mainly via MC4R, in pubertal male and female rats, even against unfavorable metabolic conditions, but not in infantile rats. Furthermore, we demonstrated that chronic blockade of MC3/4R by the administration of the antagonist, SHU9119, during the pubertal transition delayed the normal timing of puberty. On this basis, in the present work we aimed at investigating the role of α-MSH pathways in mediating the permissive effects of leptin on puberty onset and their putative interplay with the major regulator of the reproductive brain, kisspeptins, in the metabolic control of puberty. The permissive effect of leptin on puberty onset, as evidenced in female rats subjected to chronic sub-nutrition, was blunted by central inhibition of MC3/4R. In addition, analyses of a-MSH/kisspeptin interactions revealed that (a) blockade of MC3/4R by SHU9119 pre-treatment did not affect LH responses to kisspeptin, although it blocked MT-II effects; (b) selective elimination of kisspeptin receptor, Gpr54, from POMC neurons did not affect LH responses to kisspeptin; (c) POMC-Gpr54 null mice did not display overt alterations of puberty; (d) net LH responses to a-MSH agonist (MT-II), were markedly attenuated, although not totally eliminated, in Gpr54-deficient mice; and (e) blockade of α-MSH signaling suppressed Kiss1 expression in the ARC of pubertal female rats. In addition, (f) detailed neuroanatomical studies have documented close appositions between POMC and kisspeptin neurons in the ARC of pubertal female rats. Complementary electrophysiological analyses are in progress to evaluate the response of Kiss1 neurons to MT-II.

In sum, our data document the essential role of α-MSH pathways in the physiological control of puberty and in transmitting the permissive effects of leptin. While α-MSH signaling seems dispensable for the reproductive effects of kisspeptins, our findings suggest that the reproductive/pubertal actions of α-MSH are, at least partially, mediated via modulation of kisspeptin pathways.

12 Physiological Roles of Gonadotropin–Inhibitory Hormone Signaling in the Control of Mammalian Reproductive Axis: Studies in the NPFF1 Receptor Null Mouse

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RF-amide-related peptide-3 (RFRP-3), the mammalian ortholog of the avian gonadotropininhibiting hormone (GnIH), operates via the NPFF1 receptor (NPFF1R) to repress the reproductive axis, therefore acting as counterpart of the excitatory RF-amide peptide, kisspeptin (ligand of Gpr54). In addition, RFRP-3 modulates feeding and might contribute to the integrative control of energy homeostasis and reproduction. Yet, the experimental evidence supporting these putative functions is mostly indirect, and the physiological roles of RFRP-3 remain debatable and obscured by the lack of proper analytical tools and models. To circumvent these limitations, we characterize herein the first mouse line with constitutive inactivation of NPFF1R. Ablation of NPFF1R did not compromise fertility; rather, litters from NPFF1R null mice were larger than those from wild-type animals. Pubertal timing was not altered in NPFF1R deficient mice; yet, pre-pubertal KO males displayed elevated LH levels, which normalized after puberty. Adult NPFF1R null mice showed increased Kiss1 expression in the hypothalamic arcuate nucleus, higher serum FSH levels and enhanced LH responses to GnRH. However, genetic elimination of NPFF1R was unable to reverse the state of hypogonadism caused by the lack of kisspeptin signaling, as revealed by double NPFF1R/Gpr54 KO mice. NPFF1R null mice displayed altered feedback responses to gonadal hormone withdrawal. In addition, metabolic challenges causing gonadotropin suppression, such as short-term fasting and high-fat diet, were less effective in dampening LH secretion in NPFF1R deficient mice, suggesting that absence of GnIH signaling partially prevented inhibition by metabolic stress. Our data are the first to document the impact of elimination of GnIH signaling on reproductive parameters and their modulation by metabolic challenges. While, in keeping with its inhibitory role, the NPFF1R pathway seems dispensable for preserved puberty and fertility, our results surface notable alterations due to the lack of GnIH/RFRP signaling that prominently include changes in the sensitivity to fasting and obesity-associated hypogonadotropism

13 Phenotypic flexibility is influenced by the metabolically phenotypes of obesity: from the CORDIOPREV study

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Background: We examined the degree of postprandial triglyceride (TG) response over the day, representing a highly dynamic state, with continuous metabolic adaptations, among normal weight, overweight and obese patients, according to their metabolically healthy or abnormal status.
Material and methods: 1002 patients from the CORDIOPREV clinical trial (NCT00924937) were submitted to an oral fat load test meal with 0.7 g fat/kg body weight (12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates. Serial blood test analyzing lipid fractions and inflammation markers (high-sensitivity C-reactive protein (hs-CRP)) were drawn at 0, 1, 2, 3 and 4 hours during postprandial state. We explored the dynamic response according to several body size phenotypes.
Results: Metabolically healthy patients displayed lower postprandial response of plasma TG and large triacylglycerol-rich lipoproteins (TRLs)-TG, compared with those metabolically abnormal, independently whether or not they were obese (P<0.001 and P<0.001, respectively). Moreover the area under the curve (AUC) of TG and AUC of large TRLs-TG was greater in the group of metabolically abnormal compared with the group of metabolically healthy (P<0.001 and P<0.001, respectively). Interestingly metabolically abnormal subjects displayed higher postprandial response of plasma hsCRP than did the subgroup of normal, overweight, and obese metabolically healthy patients (P<0.001).
Conclusions: Our findings showed that certain types of the metabolical phenotypes of obesity are more favourable modulating phenotypic flexibility after a dynamic fat load test, through TG metabolism and inflammation homeostasis. To identify these phenotypes may be the best strategy for personalized treatment of obesity.

14 Metabolic disease, obesity and carotid atherosclerosis in patients with coronary artery disease (Cordioprev Study)

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Coronary heart disease is a major problem of health in developed countries. Although it has been suggested that obesity is a negative prognostic factor of the disease, recent data suggest that it may be the presence of an associated metabolic disease (assessed by the composite evaluation of blood pressure, inflammation and energy metabolism) which may be the true responsible of this association. Our objective was to determine the influence of isolated overweight and obesity on the carotid intima media thickness (IMT-CC), and also to assess whether this influence was determined by the presence of metabolic disease. Methods: 1002 CordioPrev study patients were studied at the begining of the study by defining their metabolic phenotypes and performing a carotid ultrasound. We analysed the influence of obesity, overweight and metabolic phenotypes in the IMT-CC. Results: The presence of metabolic disease conditioned a greater IMT-CC (0.69 ± 0.01mm vs 0.73 ± 0.01mm, p = 4 * 10-6). Overweight and normal weight patients without metabolic disease showed less IMT-CC than the groups with metabolic disease (all p<0.05). The obese participants without metabolic disease showed an intermediate behavior, without differences either with healthy and metabolically ill patients. When we evaluated only body weight, overweight or obesity by itself did not differ from normal weight patients in their IMT-CC (normal weight 0.71 ± 0.02mm, overweight 0.71 ± 0.07mm, obese 0.73 ± 0.06mm, p = 0.077). However, obesity was a determinant of IMT-CC when compared to the composite group of normal weight and overweight. Conclusions: Metabolic disease, presence of two or more metabolic abnormalities, is associated to a greater IMT-CC in coronary patients, and could be related to a higher risk for suffering new cardiovascular events. The protection conferred by the absence of metabolic disease in the IMT-CC may be blunted by the presence of obesity.

15 Evidence for the involvement of RFRP/NPFF1R system in the regulation of metabolic homeostasis: Studies in the NPFF1 Receptor Null Mouse

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RF-amide-related peptide-3 (RFRP-3), the mammalian ortholog of the avian gonadotropin inhibiting hormone (GnIH), operates via the NPFF1 receptor (NPFF1R) to repress the reproductive axis. Interestingly, the RFRP/NPFF1R system could contribute also to the integrative control of energy homeostasis and reproduction. Thus, recent pharmacological data suggest that the GnIH/RFRPs system may act as a signal of energy insufficiency, promoting food intake; an action that is likely mediated via NPY/AgRP and POMC/CART neurons in the arcuate nucleus. Yet, the metabolic dimension of GnIH/RFRP signaling in the control of metabolism and body weight (BW) homeostasis, and its eventual value as target for therapeutic intervention, remains poorly characterized.

To address the metabolic dimension of this system, we have generated the first mouse line with genetic inactivation of the canonical GnIH/RFRP receptor, the NPFF1R KO mouse. Our metabolic studies using NPFF1R KO mice revealed altered responses to high-fat diet (HFD), which were sexually dimorphic. Thus, while no difference in BW gain was observed in males between NPFF1R KO and control (WT) mice fed on HFD, a rapid increase in BW following HFD was detected in NPFF1R KO females, which exceeded that of WT animals. Likewise, ovariectomy (OVX), which is known to cause obesity due to the loss of estrogens, induced a much rapid rise of BW in NPFF1R mutants, when combined with HFD. In turn, NPFF1R null male mice fed on HFD displayed glucose intolerance (as revealed in glucose tolerance tests) that was not evident in WT mice. In addition, the RFRP/NPFF1R system appeared to be involved in mediating the effects of leptin and ghrelin on the control of food intake, because NPFF1R KO mice showed increased sensitivity to the anorectic actions of leptin and some degree of resistance to the orexigenic actions of ghrelin.

Overall, our data unveil notable metabolic actions of the GnIH/RFRP system that seem to play (partial) protective roles against obesity (following HFD and OVX) in the female and glucose intolerance (as indirect sign of insulin resistance) in the male. Our findings also suggest that GnIH/RFRP pathways may mediate, at least partially, the appetite-modulatory effects of leptin and ghrelin, the major reciprocal regulators of food intake in mammals.

16 The gut microbial community changes according to the diet in cardiovascular risk patients

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Introduction: Gut microbiota has been proposed as an additional contributing factor to the pathophysiology of obesity. In fact, studies in animal model have shown that obesity is associated with an increase in the ratio Firmicutes/Bacteroidetes, which is transmissible between individuals. The gut microbiota are involved in the regulation of body weight and associated disease obesity, acts collectively as an organ fully integrated in host metabolism, involved in energy extraction from nutrients, regulating la immunity, and participating in the control of the energy balance.

The Mediterranean diet consumption has been associated with the low rate of cardiovascular mortality found in Mediterranean countries. Moreover, the health benefits associated to the Mediterranean diet may be attributed to the phenolic compounds from virgin olive oil. In this study, we hypothesized that some of the benefits induced by the Mediterranean diet may be mediated by changes in the microbiome.

Objective: We aimed to find the changes in the microbiota composition after the long-term consumption of a Mediterranean diet and a low-fat high-carbohydrates diet in an obese population in cardiovascular risk.

Methodology: We analyzed the changes in the microbiota composition after a year of dietary intervention (Mediterranean diet (35% fat, 22% MUFA) or low-fat diet (28% fat, 12% MUFA), by pyrosequencing methods. In addition, we determined diet effects in the energy-related hormone levels and endotoxemia, and their relationship with the microbiota composition.

Results We observed an increase in Prevotella genus after the long-term consumption of the low-fat diet as compared to Med Diet (p=0.028). Moreover, the long-term consumption of the Med Diet increased the relative abundance of Roseburia and Oscillospira genera as compared to the low-fat diet (p=0.009 and p=0.016, respectively).

Conclusion: the beneficial properties associated to the Mediterranean diet consumption may be mediated, at least partially, by shaping the microbial composition of the gut

17 The association between clinicopathological characteristics and molecular phenotype could help to predict the appropriated medical therapy in human pituitary growth hormone-secreting tumors

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BACKGROUND: Acromegaly is the consequence of excessive growth hormone (GH) secretion, usually produced by a pituitary adenoma. Transsphenoidal surgery is the first-choice treatment; however, the development of new drug therapies in the last years, specially the somatostatin analogues (SSA), has open new and promising avenues for the treatment of pituitary tumors.
OBJECTIVE: To determine whether a detailed knowledge of the clinico-pathological characteristics of the acromegalic patients and the adenoma molecular phenotype could help to predict the hormonal response to therapy in order to improve the management of these patients.
MATERIAL AND METHODS: Observational study including patients with acromegaly, diagnosed at the Endocrinology and Nutrition Unit of the Hospital Reina Sofía from 2007 to 2012, in which surgery and molecular phenotyping of the adenoma was carried out.
RESULTS: 17 patients were included. Patients with acral growth significantly express more somatostatin receptor subtype-1 (SSTR1) [Z-2.36 (p=0.018)]. Furthermore, patients with arthralgia express more SSTR1 [Z-2.586 (p=0.010)] and less dopamine subtype-2 receptors (DR2T) [Z-0.037 (p=0.042)] and dopamine subtype-2 receptor long-isoform (DR2L) [Z-2.535 (p=0.011)]. An inverse correlation between insulin-like growth factor-I (IGF-I) and DR2L [Rho-0.757 (p=0.003)] was observed; as well as a direct correlation between IGF-I and left-right diameter adenoma [Rho+0.661 (p=0.014)]. The inferior-posterior diameter adenoma was directly correlated with dopamine receptor subtype-1 (DR1) [Rho+0.795 (p=0.018)] and somatostatin receptor subtype-3 (SST3) [Rho+0.615 (p=0.033)]. There was an inverse correlation between GH after surgery and Ki67 expression [Rho-0.879 (p=0.004)]. Notably, patients with biochemically controlled disease, have more expression of DR2T [Z-2.379; p=0.017] and a smaller diameter of the adenoma [Z-2.021; p=0.009].
CONCLUSION: Overall, our results indicate that there is a significant correlation between several pre-surgical clinical parameters and the molecular phenotype of GH-producing adenomas; therefore, these findings might help to predict the hormonal response to therapy and to choice the appropriate medical therapy for these patients.

18 Influence of endothelial dysfunction on telomere length in subjects with metabolic syndrome: LIPGENE study

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Aim: Previous evidences support that increased oxidative stress (OxS) may play an important role in metabolic syndrome (MetS) and both are closely linked to vascular dysfunction. The purpose of this study is to determine whether endothelial dysfunction is associated through OxS mechanisms, with the relative telomere length (RTL) in MetS subjects from the LIPGENE cohort.
Methods: In this cross-sectional study the RTL from 88 subjects (36 men and 52 women) was determined by qPCR from genomic DNA. The RTL was categorized into quartiles (shortest to longest) resulting in the following distribution, quartile 1: RTL ≤ 1.10, quartile 2: 1.10 < RTL ≤ 1.26, quartile 3: 1.26 < RTL ≤ 1.56, quartile 4: RTL > 1.56. Furthermore, we measured ischemic reactive hyperemia (IRH), total nitrite (NO) and protein carbonyl (PC) plasma levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) plasma activities.
Results: A significant increase was observed in IRH and NO plasma levels in subjects from quartile 3 ($p = 0.015$ y $p = 0.012$, respectively) and quartile 4 ($p = 0.011$ y $p = 0.016$, respectively) with longer RTL compared to subjects from quartile 1. On the contrary, PC plasma levels ($p = 0.036$) and the plasma activities of the enzymes SOD and GPx ($p = 0.003$) were lower in subjects from quartile 4 compared to quartile 1 with shorter RTL.
Conclusions: Our results suggest that endothelial dysfunction, related to high levels of OxS, could be involved in an increment of telomere attrition. Thus, further support of the molecular and cellular mechanisms involved in vascular dysfunction may contribute to the development of strategies to decelerate vascular aging or prevent cardiovascular disease.

19 Effect of exercise added to a two low calorie diets on oxidative stress in patients with metabolic syndrome

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INTRODUCTION: In the pathogenesis of metabolic syndrome, increased oxidative stress plays a key role in close relationship with endothelial dysfunction, insulin resistance and chronic inflammation. The aim of this study was to analyze the expression levels of genes involved in oxidative stress in patients with metabolic syndrome after two hypocaloric diets intervention with or without the addition of exercise.
METHODS: 40 subjects with metabolic syndrome were selected and randomized into 4 groups: 1 group with hypocaloric Mediterranean diet (MED), another with similar diet but performing physical exercise (MEDE); a third group with hypocaloric diet rich in carbohydrates (CHO) and another with similar diet but also same exercise that MEDE (CHOE). The mRNA expression levels of genes gp91phox, Nrf2 and SOD2 were determined at baseline and after the time period of 3-month intervention, using the platform OpenArrayTM multiarray .
RESULTS: The consumption of CHO diet increased the mRNA levels of gp91phox gene as compared to MED diet, independently of the physical exercise ($p = 0.004$, $p = 0.018$, respectively); the consumption of CHO diet increased the mRNA levels of Nrf2 ($p = 0.022$), and it remained unchanged when patients consumed this diet but they also performed moderate to high endurance training; by contrast, the antioxidant SOD2 gene increased ($p = 0.022$) after the performing of moderate to high endurance training when patients consumed CHO diet, but the expression of this gene remained unchanged when patients performed the same endurance training but they consumed the Med diet.
CONCLUSION: Our results suggest that physical exercise is an additional factor to the diet in the modulation of oxidative stress process in patients with metabolic syndrome

20 Fractional excretion of phosphate is increased in rats fed hypercaloric diet

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INTRODUCTION AND AIMS. Changes in mineral metabolism are always present in Chronic Kidney Disease (CKD). Abnormal mineral metabolism, and particularly the accumulation of phosphate (P), plays a central role in the generation of vascular calcifications (VC). High caloric dietary intake is a major cause of metabolic syndrome, which is associated with VC in the general population. Fibroblast growth factor (FGF)-23 is a regulator of serum phosphate and calcitriol (1,25(OH)2D3) levels. In human CKD, plasma FGF-23 appears to be a sensitive biomarker of abnormal renal phosphate handling, as FGF-23 levels increase during early stages of kidney malfunction. In this work, we evaluate the effect of feeding a hypercaloric diet on the fractional excretion of P in non uremic rats.

METHODS. After obtaining baseline blood samples, male Wistar rats (n=12) were divided in two groups: one group was fed a hypercaloric diet with 60% energy from fat (HC). The second group was fed a standard diet with normal caloric content (NC). Both diets had high phosphate content (1.2%) Seven days later, rats were housed in metabolic cages. They were fed the same HC or NC diet and during 4 days urine samples were collected to measure urine creatinine and P, and % of ingested P excreted by urine. Last day of experiment blood samples were also taken to determine fractional excretion of phosphate, PTH and FGF23.

RESULTS. Treatment Fractional excretion of P (%) % of ingested P excreted by urine

HC	48.09±4.29*?	47.49±3.44*
NC	33.31±4.47	32.25±1.36
	Plasma P (mg/dL)	Plasma PTH (pg/ml)
HC	5,00±0,377*	130,98±24.37
NC	6,27±0,192	135.68±10,36
	Plasma FGF23 (pg/ml)	
HC	440,32±59,05*	
NC	207,17±41,91	

Values are mean ±SE; *P<0.05 vs NC

CONCLUSIONS. These data show that feeding a hypercaloric diet with high fat content may increase plasma FGF-23 levels and the urinary excretion of P.

21 The expression of miRNAs are modulated by the Mediterranean diet in patients with cardiovascular disease

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Objective: To study the effect of two healthy diet (Mediterranean diet rich in virgin olive oil , and a low-fat diet) on the levels of circulating miRNAs involved in molecular mechanisms of cardiovascular disease in patients with high coronary risk.
Methods: 40 patients with cardiovascular disease, between 20 and 75 years old, were selected from the CORDIOPREV intervention study. They were asked a narrow dietary and clinical control. These patients consumed two healthy diets, a Mediterranean diet (34 % fat, 22 % MUFA , PUFA 6% and 7 % SAT) and one low-fat and high in complex carbohydrates (28 % fat, 12 % MUFA , 8% and 8 % PUFA SAT) diet at randomization. The extractions of blood samples were performed at baseline and after the first year of dietary intervention. The levels of miRNAs circulating in plasma were isolated from each patients and the expression of miR-210a and miR-30a-3p were analyzed by RT- qPCR. Statistical analyses of the data were performed with SPSS 15.0.
Results: The expression of miR-210a increased after consumption of the Mediterranean diet, compared with the low-fat diet in patients with cardiovascular disease and metabolic syndrome ($p = 0.035$). However, we didn't observe significant differences in patients without metabolic syndrome. The comparative analysis between the two types of diet in patients with cardiovascular disease and diabetes showed an increased expression of miR30a-3p, after consumption of the Mediterranean diet ($p = 0.022$). By contrast, we didn't observe significant differences in patients with cardiovascular disease without diabetes.
Conclusion: The Mediterranean diet modulates the expression levels of miR-210a and miR-30a-3p, considering them as potential therapeutic targets in patients with cardiovascular disease and diabetes and with metabolic syndrome.

22 Socioeconomic factors and adherence to the Mediterranean diet. Preliminary results. Cordioprev study.

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Introduction : The typical Mediterranean eating pattern has a positive effect on secondary prevention in patients with cardiovascular disease (CVD). Socioeconomic factors (ESF) could determine the level of adherence to this dietary pattern .
Objective : To analyze the ESF of a population in secondary prevention of CVD (ischemic heart disease and / or ischemic cerebrovascular disease) and adherence to a Mediterranean diet (DMED). Analyzing cardiovascular risk factors and foods comprising the DMED key according to the FSE population .
Methods: Cross-sectional study in 760 individuals selected secondary prevention of CVD CORDIOPREV study (Dietary Coronary Prevention Study with Olive Oil) , aged between 33 and 76 years. All subjects underwent an adherence questionnaire of 14 questions to Mediterranean Diet and a socioeconomic questionnaire . It population ranked the low adherence to Mediterranean Diet (BAMed) score <9 , or high adhesion DMED (ALMED) score ≥ 9. The following ESF study groups.
Results: The average rating adherence questionnaire Mediterranean Diet of the total population, shows a BAMed to this dietary pattern (8.57 ± 2.24) .
The BAMed group , had a higher proportion of cardiovascular risk factors , less educated , most lived in rural areas and had a lower income level.
Conclusion : There is a higher proportion of individuals with low socioeconomic status among patients in secondary prevention of CVD that have BAMed and worse prognosis in cardiovascular risk factors .

23 In1-ghrelin splicing variant increases malignant features in breast cancer cell lines

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Ghrelin (GHR) is a pleiotropic hormone encoded by a gene that originates several splicing variants, which are associated with multiple pathophysiological functions. Specifically, our group identified a novel, functional, human splicing-variant named In1-ghrelin, which is originated by the retention of intron-1. Interestingly, this variant was found overexpressed in a battery of primary breast cancer samples compared to normal mammary glands and its expression correlated with the expression of proliferation markers, suggesting an implication of In1-ghrelin in breast cancer progression and development. However, the role of In1-ghrelin in the modulation of cancer-associated events such as tumor cell proliferation, migration or epithelial-mesenchymal transition (EMT), are still unknown. Therefore, the present study was aimed to determine the functional consequences of the overexpression of In1-ghrelin variant in breast cancer cells and to determine whether overexpression of native-GHR exert different functional consequences to In1-ghrelin in these cells. Therefore, we developed monoclonal cell lines stably-transfected with In1-ghrelin, GHR or the empty vector (MOCK) in the aforementioned cell line (MDA-MB-231) and different cancer-associated parameters including cell proliferation (Alamar blue assay), cell migration (wound healing assay) or EMT (determining number of cells with mesenchymal-like phenotype) were analysed. These studies demonstrated increased proliferation rates (p=0,013 at 72-hours) and migration ability (p=0,002) in the In1-ghrelin cells compared with MOCK- or GHR-cells. In addition, a higher number of cells with mesenchymal-like phenotype were found in In1-ghrelin transfected cells (p=0,0136), where P-Cadherin expression was reduced (typical in EMT processes), as compared to GHR- and MOCK-cells. Altogether, our results suggest that In1-ghrelin might play a relevant role in breast cancer malignancy and, therefore, it might be used as a diagnostic, prognostic and/or therapeutic marker in breast cancer based in the profound impact of In1-ghrelin overexpression in MDA-MB-231 cell line, which is clearly different, and even opposite, to that induced by native-GHR overexpression.

24 Identification of new non-invasive biomarkers for the diagnostic, prognostic and management of prostate cancer

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Prostate cancer (PCa) is an endocrine, heterogeneous and complex pathology with a high incidence in the male population. Nowadays, measurement of plasma prostate-specific antigen (PSA) is one of the most common parameters used for the diagnosis of different types of PCa; however, the diagnostic and prognostic value of PSA levels is limited in that advanced prostate cancer can be associated with very low or normal PSA values. Therefore, in order to find new, sensitive and specific, markers and tools for the diagnosis/prognostic/management of PCa, we are developing a unique, multidisciplinary, coordinated, translational (clinical-pathological-basic) initiative aimed at identifying minimally invasive biomarkers to diagnose and manage PCa. Specifically, the aim of our group in this project was to develop and validates a standardized system, using quantitative real-time RT-PCR, for obtaining a molecular phenotype of several potential biomarkers of disease in primary prostate tissue (biopsies) from individuals with PCa. Specifically, molecular information of 40 PCa and 9 normal samples were obtained and, clinical data of each patient was also collected (e.g. presence of metastasis, Gleason score, etc). Initial analysis indicates that several potential markers are overexpressed in PCa (several components of the somatostatin and ghrelin regulatory systems, Androgen Receptor-1/2, PCA3, etc.) compared to normal prostate tissue, which provide novel information on specific receptors known as drug-targets and new potential biomarkers that might be altered in PCa and measured in non-invasive samples (blood and urine). Based of these analyses, we are analyzing additional potential markers as well as molecules related with the regulation of these genes altered in the PCa biopsies (e.g. miRNAs). In addition, we are starting to validate the usefulness of these novel biomarkers in non-invasive samples in order to confirm their dysregulation in patients with PCa as compared with controls and, consequently, their potential use as non-invasive biomarkers for the diagnostic and prognostic of PCa.

25 Human sweat metabolomics for lung cancer prediction

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Lung cancer is the carcinogenic disease with the highest mortality rate owing to the advanced stage at which it is usually detected; therefore, biomarkers for lung cancer detection at early stages are needed. On the other hand, sweat has recently gained popularity as a potential tool for diagnostics and biomarker monitoring.

A two-step research has been developed:

First, an analytical method for analysis of human sweat by liquid chromatography–mass spectrometry (LC–Q-TOF MS/MS) in high resolution mode was developed. Forty one compounds were identified by the MS/MS information obtained with a mass tolerance window below 4 ppm. Amino acids, dicarboxylic acids and other interesting metabolites such as inosine, choline, uric acid and tyramine were identified.

The second step was to use the developed method for analysis of sweat to discriminate between patients with lung cancer versus control individuals (smoker and non-smoker individuals). The capability of the metabolites identified in sweat to discriminate between both groups was studied. Among them, a trisaccharide phosphate presented the best independent performance in terms of the specificity/sensitivity pair (80 and 72.7%, respectively). Additionally, two panels of metabolites were configured using the PanelomiX tool as an attempt to reduce false negatives (at least 80% specificity) and false positives (at least 80% sensitivity). The first panel (100% specificity and 63.6% sensitivity) was composed by nonanedioic acid, γ-GluLeu dipeptide and maltotriose, while the second panel (88.6% specificity and 81.8% sensitivity) included nonanedioic acid, maltotriose and the monoglyceride MG(22:2). As both panels were supported on high sensitivity and specificity values, the proposed approach would be based on the analysis of the four implicated metabolites. The combined use of the two panels would allow reducing the number of cases subjected to confirmatory test with the minimum rate of false negative and positive rates (0% and 18.2%, respectively).

26 Metabolomic Profiling and Fingerprinting of Human Lung Adenocarcinomas

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Background: Lung cancer is the leading cause of cancer related mortality worldwide. The prognosis is very poor, with an overall 5 years survival of 10 to 15%. Adenocarcinoma is the most common histologic type of lung cancer. The aims of this study were to assess whether lung adenocarcinoma samples reflect a distinct metabolomic profile compared with normal tissue from the same patients, and the potential use of dominant metabolites for new therapies or to improve lung cancer diagnosis.
Methods: Between January 2011 and December 2012, fresh samples of lung adenocarcinoma tumor tissue and the surrounding healthy lung were obtained from 33 patients following curative intent lung resection. The extracted samples were split into equal parts for analysis on the GC/MS and LC/MS/MS platforms. The biochemical profiles of normal and tumor tissue from the same individuals were compared. Welch's two-sample t-test was used to identify biochemicals that differed significantly between both groups. Screening for excreted metabolites with potential to bind TRP receptors was performed.
Results: A total of 271 metabolites were significantly altered in lung cancer samples compared to their corresponding healthy lung tissue ($p<0,05$). Among them, 223 were significantly induced, as opposed to only 48 that were significantly diminished. Alterations in major metabolic pathways as Lipid oxidation, Nucleotide and Arginine metabolism and Redox homeostasis have been identified. Approximately 50 of these metabolites, involved in different metabolic pathways, show high affinity for TRP receptors.
Conclusions: Lung adenocarcinoma exhibits alterations in glucose utilization that may support the synthesis of nucleic acids and anabolic metabolism, but also contribute to the generation of harmful glycation products. Disruptions in lipid oxidation were also observed and may reflect altered mitochondrial function. Furthermore, differences in arginine and phospholipid metabolites may be indicative of tissue remodelling in these samples, while enhanced one carbon metabolism and higher ADAM levels may contribute to altered lung function and the aggressiveness of lung cancer. Additionally, some of these metabolites with affinity for TRP receptors may play a regulatory role in the initiation and progression of lung cancer. Reversal to normal levels of some metabolites may provide a target for the development of next-generation anticancer agents targeting tumor metabolism.

27 The E3 Ubiquitin ligase SIAH2 is a central regulator of DYRK-family kinases class II

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The E3 ubiquitin ligase SIAH2 is a key protein in the regulation of important processes such as hypoxia, inflammation and carcinogenesis. A large number of SIAH2 substrates implicated in carcinogenesis have been described to date, among which the hypoxia-regulating family of prolyl hydroxylases (PHDs), Promyelocytic leukemia protein (PML) or Homeodomain-interacting protein kinase 2 (HIPK2) are included. We previously described the mutual regulation between SIAH2 and the dual-specificity tyrosine phosphorylation regulated kinase 2 (DYRK2), which play a very important role in the control of tumorigenesis. For this reason, the objective of this study was to analyze the SIAH2 role on the regulation of the rest of kinases from the same family, DYRK3 and DYRK4.

In this work, we show for the first time that DYRK3 phosphorylates SIAH2 at least in four residues (Thr26, Ser28, Ser68 y Thr119), whereas DYRK4 is able to increase the phosphorylation in only one residue (Ser28). Phosphorylated SIAH2 by DYRK3, and not by DYRK4, is more active than the wild-type E3 ligase and facilitates PHD3 polyubiquitination, a relevant protein in the hypoxia response control. Co-immunoprecipitation experiments show DYRK4 as SIAH2 interaction partner, failing to observe this interaction with DYRK3. On the other hand, we also show that SIAH2 facilitates DYRK3 and DYRK4 ubiquitination and degradation, being the first ubiquitin ligase described to date for these two kinases.

These results are particularly relevant. First, we show two new kinases able to phosphorylate and modulate the SIAH2 activity. Furthermore, we show SIAH2 as master regulator of class II DYRK family kinases, which together with its oncogenic functions, confirm the potential clinical applications of targeting SIAH2 in cancer therapy. The discovery of proteins like DYRK3 and DYRK4 with new roles in carcinogenesis opens new strategies for future therapies.

28 Workload and performance evaluation using 3D vision versus 2D vision in subjects inexperienced with laparoscopy

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Objectives: To appraise the effect of vision in three dimensions (3D) compared with two dimensions (2D) on the laparoscopic performance and supported workload in simulation exercises.
Methods: A prospective randomised cross-over study on students inexperienced with laparoscopy was conducted. The participants completed five pelvitrainer exercises based on a validated program using both vision systems. The participants' performance was measured by their time (in seconds) and the number of errors they committed during the procedures. The workload was assessed by the validated NASA-TLX questionnaire. Depth perception, image quality, visual discomfort and headache were measured on a scale of 0-20.
Results: Forty-six participants were included in the analysis. The participants performed better using 3D vision in terms of time (3D= 1006.08±315.94 vs 2D= 1309.17±300.28; p< 0.001) and in the total number of errors (3D= 0.84±1.26 vs 2D= 1.86±1.60; p< 0.001) in executing the exercises. This use of 3D vision also exhibited superior times when the tasks were evaluated independently: I) "transfer objects" (p= 0.001), III) "single knot" (p< 0.001), IV) "clip and cut" (p< 0.05), and V) "needle guidance" (p< 0.001).
Furthermore, the use of 3D vision produced less of a workload, according to the NASA-TLX results (3D= 53.89±13.08 vs 2D= 62.10±12.59, p< 0.001), although its use was associated with greater visual discomfort (p< 0.01) and headache (p< 0.05).
Conclusions: Incorporating 3D systems into laparoscopic surgery would facilitate the learning curve because it produces a lower workload that is accompanied by an elevated performance.

29 Gene-expression profile of CD3+Lymphocytes from patients with chronic graft versus host disease after allogeneic stem cell transplant under immunosupresive treatment

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INTRODUCTION: Chronic graft-versus-host disease (cGVHD) is a main cause of morbility and non-relapse mortality in patients who underwent allogeneic stem cell transplantation (allo-SCT). The key role of donor-derived T lymphocytes in triggering the harmful immune response in acute GVHD is clear but the implication and activatin mechanisms in the development of cGVHD are less understood. Analysis of transcritpome by mean of Gene expression profile might provide a stepforward to identify involved activatioN pathways and to undercover potential therapeutic targets
PATIENTS AND METHODS: A total of 18 patients with a follow-up longer than 100 days after allo-SCT were transversally recruited. Donor type was HLA_identical sibling donor (N=14) or Unrelated Donor (N=4). Stem cell sources were Bone marrow (N=9), mobilized peripheral blood (N=8) or Umbilical cord (N=1). At time of analyses 10 patients had developed limited or extense cGVHD being under immunosupresive drugs = Prednisone (PRD) (N=2) , CsA + P (N=6) or FK+PRD (N=2) while 8 patients remained without symptoms of cGVHD. Ten healthy donors were also enrolled as controls. Purification of CD3+ peripheral blood lymphocytes were performed with AutoMACS and RNS extraction were performed by RNAeasy (Quiagen). After reverse transcription cDNA were hibridized using GeneChip plattform (Affymetrix). After signal normalization with RMAExpress, ssignificance analysis of microarray (SAM) was performed to identify the differentially expressed genes in GVHD patients with a false discovery rate (FDR) level of 5% were obtained. Ingenuity Pathways Analysis software were used to assess the functional composition of genes and related regulatory networks. RESULTS: Non-supervised analysis samples from patients with or without cGVHD and normal subjects are grouped. SAM analyses showed a differential upregulation of 1570 genes in patients without cGVHD compared to controls including those involved in MAPK activation, angiogenesis, mRNA splicing machinery, T-cell citotoxicity and DNA repair. Immunosupressive agents causes a down-regulation of several genes. Thus, SAM analyses showed 792 upregulated genes in treated-patients with cGVHD compared to healthy subjects including thise involved in MAPK activation and cytotoxicity and immune response. Comparing treated-patients with cGVHD to patients wihtour GVHD, the most significantly downregulated genes included : JUN, SOX4, MAPkinases (MAP3K14, MAP4K5, MAPKAPK3), MDM4, cyclones and kinase-dependent cyclins. Impaired signaling pathways induced by immunosuppressive treatment in T lymphocytes from patients with cGVHD included EIF2 eEIF4 (transduction inducers), granzime-A, docosahexanoic acid, (DHA), JAK/Stat and ErbB2- ErbB3. It is noteworthy the down-regulation of IL-2, IL-3, IL-4, IL-15, IL-17, IL-22 after immunosuprression. CONCLUSSION: GEP of purified CD3+ donor lymphocytes in patients alter allo-SCT with cGVHD under immunosuppressive treatment allow the identification of a significant number of down-regulated signaling pathways compared to patients without cGVHD including Interleukins production and JUN, SOX4 and MAPKinases.

30 Rehabilitation of erectile dysfunction after radical prostatectomy

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Objective: To evaluate the results in the treatment of erectile dysfunction postprostatectomy through the use of vasoactive drugs.
Material and method: a retrospective study on 82 patients treated with intracavernous injections after radical prostatectomy. The inclusion criteria were patients not responders to 5-Phosphodiesterase inhibitors without a history of erectile dysfunction prior to the intervention. The patients were divided into two groups depending on if the beginning of the treatment: early (≤6 months) or late (>6 months). Following injection of prostaglandin E1 20 mg., held color ecodoppler penile in all patients. Values considered as normal were: a peak systolic velocity (PSV) ≥ 30 cm/sec. and the end diastolic velocity ≤ 5 cm/sec.
Results: 82 patients evaluated, 44 began the rehabilitation of the corpora cavernosa in the 6 months after surgery. In the multivariate analysis, were factors predictive of good response to treatment: early onset of rehabilitation (OR:0,06; 95%CI: 0,014-0,26), higher PSV during the test (OR:1,01; 95%CI: 1,01-1,1) as well as a pro-histopathologic Stadium (OR:0,15; 95%CI: 0,036-0,6).
Comparing hemodynamic status according to the beginning of rehabilitation, the color ecodoppler of the penis following injection of PGE1 presented abnormal values with greater frequency in the group of late onset compared to the early: 89,5% (n=34) vs. 65,9% (n=29), respectively (p=0,01).
The Doppler showed abnormal values in 63 of the patients (77%), being more frequent in front of the early late-onset group (89,5% [n=34] vs. 65,9% [n=29]; p=0,01). The 40,2% (n=33) presented failure of the mechanism of corporovenooclusivo with mean values of 5,11±1,3 cm/sec., featuring higher values the late-onset group (5,53±1,4 cm/sec) against the early (4,75±1,03 cm/sec) (p=0,005).
Conclusions: According to this study, early erection drug rehabilitation is best compared to the late results, being the color ecodoppler a valuable tool in the diagnosis and prognosis of response to treatment in patients postprostatectomy.

31 Fibroblast growth factor 23 downregulates osteogenic markers in mesenchymal stem cells derived-osteoblasts by inhibition of Wnt/beta-catenin signaling pathway

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In chronic kidney disease, patients often undergo loss of bone mineral density leading to bone disorders. Uremic patients show very high levels of serum fibroblast growth factor 23 (FGF23) a phosphaturic hormone secreted by osteoblasts and mature osteocytes. High plasma concentrations of FGF23 are associated to mortality, left ventricular hypertrophy, vascular calcification and other changes such as inhibition of phosphorus reabsorption, activation of calcium reabsorption, inhibition of PTH secretion and 1,25-dihydroxivitaminD3. We checked that the exposition of mature osteoblasts derived from mesenchymal stem cells (MSC) to uremic serum from nephrectomized rats led to a down-regulation of osteogenic markers. The question is whether high levels of FGF23 from uremic serum could be responsible of these changes. Therefore the effects of high FGF23 were evaluated on immature and mature osteoblasts.

High levels of FGF23 were evaluated during the osteoblasts differentiation process of MSC. Finally the effects of high levels of FGF23 were studied on mature osteoblasts obtained by MSC differentiation for 21 days. Results show that high levels of FGF23 during the osteoblast differentiation did not modify ostegenesis and mineralization. However, on mature osteoblasts 12 hours or high FGF23 levels led to a down-regulation of early osteogenic markers such as Osterix and Osteocalcin and mature osteoblast markers as DMP1, Osteoprotegerin and importantly RANKL. These effects were associated to an increase of sclerostin and a decrease of nuclear translocation of beta-catenin. The administration of a monoclonal antibody against FGF23 in presence of high levels of FGF23 led to a decrease of sclerostin, beta-catenin stabilization and an increase of osteoblast and osteocytes markers.

In conclusion, in mature osteoblasts high levels of FGF23 decrease the expression of osteoblast and osteocytes master genes by inhibition of canonical Wnt signaling. Therefore high levels of FGF23 are one of the factors which an adynamic bone may be induced in uremic patients.

32 Clinical and epidemiological features of patients with inflammatory bowel disease and extraintestinal manifestations associated

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Background Inflammatory bowel disease (IBD) primarily affects the gastrointestinal tract, but throughout its evolution, patients may experience extraintestinal involvement. We aimed to describe the clinical and epidemiological characteristics and the extraintestinal manifestations (EIM) on IBD in our community. Methods A retrospective and case–control study was performed. We identified the ulcerative colitis (UC) and Crohn's disease (CD) patients with EIM (114 cases) from Reina Sofia Hospital and randomly matched each case with two IBD patients without EIM (a 228 contrcontrols).. Demographic and epidemiological data were collected. Results We analyzed 342 patients with a mean age of 45.9 years (SD: 13.3) (cases) and 45.4 years (SD: 13.1) (controls). Mean age at IBD diagnosis: 33.6 years (SD: 14.1) and 35.6 years (SD: 14.4) respectively, with no significant differences by type of IBD. In both groups, 50.8% were female, 36.5% patients with UC and 68.4% with CD. Proctosigmoiditis in UC and ileocolic disease in CD were the most frequent location. Extraintestinal involvement: osteoarticular (48.2%), muco-cutaneous (30%), ocular (16.4%), haematologic (2.8%) and biliary manifestations (1.4%). Most frequent EIM: erythema nodosum in CD (79.1%), sacroiliitis and spondylitis in UC (73.0%) ($p = 0.05$). We identified higher prevalence of EIM at diagnosis of IBD in CD (22.3%) vs UC (11.6%) ($p = 0.09$). Factors associated with the development of EIM were: ileocolic location in CD ($p = 0.04$), presence of granulomas in CD biopsy ($p = 0.002$) steroid-dependence ($p = 0.01$) and use of biological therapy ($p = 0.02$). Conclusion The osteoarticular and mucocutaneous manifestations were the most frequent EIM. Ileocolic location and presence of granulomas in CD, steroid-dependence and needed for biological therapy were associated to increased risk of EIM. Further studies on larger cohort of patients are required to develop a predictive model to identify risk patients and to treat them early.

33 Skeletal muscle histology in an experimental relapsing model of multiple sclerosis

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Introduction. Skeletal muscle is a target organ in multiple sclerosis, a neurodegenerative disease, in which among 65-97 % of patients have neuromuscular symptoms. Given the lack of information about skeletal muscle pathology, our objective was characterizing skeletal muscle histology in the experimental autoimmune encephalomyelitis model of relapsing-remitting multiple sclerosis.
Methods. 16 Dark Agouti rats were divided in four groups: normal control (any intervention), vehicle control (Freund's adjuvant) and experimental autoimmune encephalomyelitis (induction with MOG + Freund's adjuvant) animals sacrificed at the onset of the disease and at the end of the relapsing phase. Extensor digitorum longus (EDL) and soleus muscles were removed. Both electron microscopy and light microscopy samples were taken. Histological and histochemical stains were performed on cryostat cuts. ImagePro Plus 4.5 and SigmaStat 3.1 were employed for morphometric and statistical analysis, respectively.
Results. At the onset of the disease, we found ragged-red fibers and degenerating mitochondria in both muscles, without signs of atrophy. At the end of the relapsing phase, a cross-sectional area reduction and a decrease in the minor diameter of fibers were detected. There were also intermediate fibers with ATPase pH 9.8 in both muscles. Histochemically the appearance of core-targetoid cytoarchitectural changes in soleus differed from the reduction in NADH-tr stain in EDL. Ultrastructurally, there were myofiber disarray, degenerating mitochondria and autophagic vacuoles containing mitochondria.
Discussion. Histological features at the onset of the disease resemble mitochondrial myopathy, whereas those at the end of the relapsing phase point to a denervating process. The mitochondrial alterations found in our model could be in relation with the growing evidence for a mitochondrial degeneration in multiple sclerosis and experimental autoimmune encephalomyelitis. In this case, skeletal muscle could be used as a target organ to study the effect of therapies in experimental autoimmune encephalomyelitis.

34 Ground reaction forces in patients with knee osteoarthritis after intra-articular treatments.

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Osteoarthritis of the knee (OA) is the most common disease affecting the musculoskeletal system and one of the most important causes of disability among elderly population. Patients are assessed by questionnaires whose response may be influenced to some subjectivity (WOMAC, VAS, SF36). Gait analysis and ground reaction forces (GRF) produce objective and quantitative results of patient status with well-defined patterns. Vertical force curves are characterized by showing two peaks and a valley, showing a graph like an inverted W. Intra-articular injections of hyaluronic acid for the treatment of knee OA have been shown to reduce pain and improve joint function. In this work we have used GRF as evaluation tool in patients with knee OA before and after applying intra-articular treatments.

35 Role of uremic toxin P-Cresol in the Vascular Endothelium: A proteomic approach

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The vascular endothelium is one of the largest organ and most important of the human body. Their structural and functional integrity are essential to maintain homeostasis of the vascular wall and circulatory function. Endothelial dysfunction is closely associated with chronic renal failure. Some uremic toxins such as p-cresol present in the different stages of CKD, injury the endothelial cells, and induce an endothelial dysfunction.

In our study, we used HUVEC as a model of human vascular endothelial cells and we assesment the molecular mechanisms that are involved when they are in contact with P-Cresol. Know the ''proteome'' and changes in general protein expression in these cells representative of the endothelium, could give us an idea of what would happen in vascular endothelial cells when exposed to P-Cresol.

We identified 14 differentially expressed proteins when cells are treated with P-cresol, 6 of which are expressed only in the presence of the toxin. Moreover, we have observed that many of these are related to the cytoskeleton, oxidative stress proteins and protein basal metabolism.

We have also observed significant differences in protein expression when cells have not been exposed to the toxin. Our data suggest the hypothesis that endothelial dysfunction caused by the p- cresol start with a destabilization of the cell monolayer , following more complex processes that need to be studied further.

Applied to the clinical process , we could relate this difference in protein expression with endothelial cells of patients, to even be able to find endothelial biomarkers that provide early signals about the endothelial injury of this toxin, always within the context of chronic kidney disease.

36 Study of oxidative stress in a model of experimental autoimmune encephalomyelitis: Natalizumab and dimethyl fumarate

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The multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) that affects approximately over two million people worldwide of which, 40,000 are Spanish. The reference treatment is Natalizumab, a monoclonal antibody that inhibits the migration of systemic immune cells to the central nervous system shown also to antioxidant effects. The aim of the study was to compare the antioxidant effect to Natalizumab and dymetil-fumarate, an ester of fumaric acid with anti-inflammatory and antioxidant properties. It was induced an experimental autoimmune encephalomyelitis (EAE) model by myelin oligodendrocyte glycoprotein (MOG) in Dark agouti rats. The EAE was characterized by an increase in the clinical score, together with an enhancement in oxidative stress biomarkers (lipid peroxidation products and carbonylated proteins) and reduction in GSH/GSSG ratio. In conclusion, Natalizumab and DMF decrease the clinical score caused by EAE and showing an antioxidant effect.

37 Atherosclerosis and Cardiovascular Disease in Systemic Lupus Erythematosus. Effects of in vivo Statins treatment.

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Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Objective: Statins may have beneficial vascular effects in systemic lupus erythematosus (SLE) beyond their cholesterol-lowering action, although involved mechanisms remain incompletely understood. We investigated the potential mechanisms participating in the efficacy of fluvastatin in preventing aterothrombosis in SLE.

Methods: Eighty five SLE patients and 62 healthy donors were included in the study. Selected SLE patients (n=27) received 20 mg/day fluvastatin for one month. Blood samples were obtained before the starting and at the end of treatment. Monocytes from 5 SLE patients were treated in vitro with fluvastatin.

Results: Increased prothrombotic and inflammatory parameters were found in SLE patients. SLE monocytes displayed altered mitochondrial membrane potential and increased oxidative stress. Correlation and association analyses demonstrated a complex interplay among autoimmunity, oxidative stress, inflammation, and increased risk of atherothrombosis in SLE. One-month fluvastatin treatment of SLE patients reduced SLEDAI and lipid levels, oxidative status, and vascular inflammation. Array studies on monocytes demonstrated differential expression in 799 genes after fluvastatin treatment. Novel target genes and pathways modulated by fluvastatin were uncovered, including gene networks involved in cholesterol and lipid metabolism, inflammation, oxidative stress and mitochondrial activity. Electron microscopy analyses showed increased density volume of mitochondria in monocytes from fluvastatin-treated SLE patients, which also displayed higher expression of genes involved in mitochondrial biogenesis. In vitro treatment of SLE monocytes confirmed the results obtained in the in vivo study.

Conclusion: our overall data suggest that fluvastatin improves the impairment of a redox-sensitive pathway participating in processes that collectively orchestrate the pathophysiology of atherothrombosis in SLE.

38 Biosafety and potency evaluation of mesenchymal stromal cell engineered to secret VEGF in hind limb isquemia mouse model

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Group: GC14 *Cell Therapy*

BACKGROUND: Critical limb ischemia (CLI) is a severe blockage in the arteries of the lower extremities, which markedly reduces blood-flow. Left untreated, CLI will result in amputation of the affected limb. Currently, there are no effective treatments for these no-option patients. Vascular endothelial growth factor (VEGF) induces vascular growth; in addition, it is known that bone-marrow-derived-mesenchymal-stromal-cells (MSC) migrate into hypoxic tissues promoting revascularization. A potential candidate to treat no-option CLI could be MSC engineered to secrete VEGF (MSC/VEGF). Our long-term goal is to initiate a Phase I clinical trial to treat no-option CLI patients.
AIM: Carry out a preclinical study to verify, in a hind limb-isquemia-mouse-model, if MSC/VEGF-therapy agent is safe and highly potent in secreting VEGF.
METHODS: Human BM-derived-MSC were transduced with VEGF-Lentiviral-vector or Luciferase-lentiviral-vector for:
1.Biosafety: 1a) Determination of VEGF-gene copy number in MSC/VEGF by qPCR. 1b) In vitro suicide assay: MSC/VEGF were exposed to Ganciclovir (GCV) and cell viability was analized at days 2, 5, 7 and 9. 1c) Biodistribution studies in hind-limb and control mice were realized by bioluminescence and qPCR. Immune-deficient-mice (NOD/SCID/IL2rg -/-) were injected with 1x106 luciferase-vector-transduced-MSC and tracked by bioluminescence at 48h, 7d and 15d after injection. For qPCR biodistribution 1x106 MSC/VEGF were injected and detected by Human-Thyr-gene
2.Potency: Supernatants of MSC/VEGF cultures were collected 3 days after transduction and VEGF was quantified by ELISA.
RESULTS: The number of VEGF-vector integrations/cell was within the established (≤2 integrations/cell). Gen suicide assay demonstrated that at day 9 after GCV exposition 92% MSC/VEGF were killed. Biodistribution assays showed that 7d after injection, MSC/VEGF were still present in heart and lungs (≤0.01%). No MSC/VEGF signal was found at 15d. ELISA showed that VEGF secretion in MSC/VEGF was higher than non-transduced-cells.
CONCLUSION: Our results demonstrated that MSC/VEGF-therapy agent is safe and displays high potency in producing VEGF.

39 Chronic ischemic heart disease affects bone marrow progenitor cells number and function more severely than idiopathic dilated cardiomyopathy

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Group: GC15 *Invasive Cardiology and cell therapy*

OBJETIVE. We investigated whether the number and functional capacity of progenitors present in the bone marrow-derived mononuclear cells (BM-MNCs) of patients with chronic ischemic heart disease (ICM) and idiopathic dilated cardiomyopathy (DCM) are affected in the same extent.

BACKGROUND. Selective impairment of BM-progenitors function is associated with ICM but it is not clear if the same changes occur in non-ischemic-DCM.

METHODS. We assessed the number, as well as the functional and differentiation capacities of BM-progenitors from 19 ICM-patients, 27 DCM-patients and 10 healthy-controls (Controls). The number of stem/progenitor-cells was determined by the expression of CD34, CD34/c-Kit and CD133, and functional capacity by colony-forming-unit (CFUs) ability of BM-MNCs. Expression of CXCR4 induced by high concentration of stromal-cell-derived factor-1α (SDF-1α) was determined by flow cytometry and migratory response by transwell assay.

RESULTS. The percentage of CD34+, CD34+/c-Kit+ and CD133+ cells as well as the CFUs-capacity were significantly lower in both groups of patients in comparison with controls, and in ICM-patients in comparison to DCM-patients.

The migratory ability of BM-MNCs in both groups of patients is similar and significantly higher than controls, which correspond with a higher increase in the expression of CXCR4 in these patients in response to sdf-1.

BM-MNCs	ICM Patients	DCM Patients	Controls			
%CD34+	2.03±1.25	3.77±1.59	6.31±1.67			
P-Value	<0.001 (vs Controls)	0.002 (vs Control)	<0.001 (Patients)			
%CD34+/CD117+	1.04±0.97	1.87±1.03	4.75±1.75 P-Value	<0.001(vs Controls)	0.001(vs Controls)	0.003(Patients)
%CD133+	0.08±0.11	1.83±0.828	1.98±0.98 P-Value	<0.001(vs Controls)	NS(vs Controls)	<0.001(Patients)
Cfu-Hill (x10-6)	3.33±2.21	4.41±5.06	21.9±8.55 P-Value	0.001(vs Controls)	0.004(vs Controls)	NS(Patients)
%CXCR4+ pre-migration	1.9±1.76	2.58±2.43	3.25±0.72 P-Value	0.05(vs Controls)	NS(vs Controls)	NS(Patients)
%CXCR4+ post-migration	5.15±3.53	4.28±4.17	2.95±0.03 P-Valeu	0.05(vs Controls)	0.05(vs Controls)	NS(Patients)
Increase %CXCR4+	3.254	1.706	-0.3			
%migrated cells to SDF-1α	13.28±4.06	13.62±7.37	6.82±6.06 P-Value	0.008(vs Controls)	0.008(vs Controls)	NS(Patients)

CONCLUSION. ICM affects the number and functional ability of BM progenitors more severely than DCM.

40 C. elegans as a model in preclinical assays for pharmacogenetic studies of risperidone and aripiprazole.

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Patients with a diagnosis of autism spectrum disorders (ASD) may have associated comorbidities such as irritability, aggressiveness and self-harm. Aripiprazole and risperidone, both classified as atypical antipsychotic drugs, are the only Food and Drug Adiministration approved medications for treating irritability/aggression in ASD. The drugs show different efficiency and varying side effects depending on the patients. The causes of these differences are unknown. Risperidone is a dopamine D2 and serotonin 5-HT2A receptors antagonist. Aripiprazole is hypothesized to function as an agonist or antagonist of D2 receptor, a partial agonist of the 5-HT1A and an antagonist of 5-HT2A serrotonin receptors. In this scenario, the establishment of animal models for the study of biological mechanisms of drug targets becomes fundamental. Our group has used sucessfully C. elegans as a pharmacogenetic model of fluoxetine and methylphenidate. In the present study the nematode was used for studying comparative mechanisms caused by risperidone and aripiprazole. We observed that risperidone and aripiprazole were able to alter the behavioral pattern of the worm, including the gentle touch response and the pharyngeal pumping rate. These effects remained in successive generations in both drugs indicating that epigenetic mechanisms could be involved. The different patterns of transgenerational impairments on the behavior os C. elegans indicate that the mechanisms that produce this effect may be different in both drugs. On the other the effect of risperidone and aripiprazol were different in mutants deficient in dopamine and serotonin receptors orthologs to human D2 and 5-HT receptors. These receptors belong to the super-family of seven-transmembrane G-protein-coupled receptors. The present study will likely provide novel insights on the molecular neurobiological mechanism of both drugs and will contribute to the knowledge concerning the efficacy and safety profiles of these medicaments.

41 Adhesion to a vaccination program previously established in patients with inflammatory bowel disease

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Introduction: The implementation of vaccination programs in patients with inflammatory bowel disease (IBD) is heterogeneous , so it is not well known accession of our patients with IBD to a vaccination program previously established
Objectives: Determine adherence to a vaccination program established in our center in patients diagnosed with IBD and to define what factors may predict a low commitment.
Material and methods: All patients were referred to Preventive Medicine with diagnosis of IBD between January and March 2012 , in order to determine their immune status (analytical with serology for viral hepatitis, varicella- zoster, rubella and measles).Then be vaccinated considering that immunization and their vaccination schedule . The percentage of adherence and non-adherence to the program as well as the factors associated with low adherence was determined.
Results: 153 patients (45.1 % men and 54.9 % women , mean age 43.30 ± 14.19 years) diagnosed with IBD (50.3 % 49.7 % ulcerative colitis and Crohn's disease) were included). Adherence to the vaccination program was 84.3 %. Factors that were associated with poor adherence were type of medication (immunosuppressive and/or biological presented greater adhesion versus those not taking p 0.021) , adherence to medical treatment (those with poor adherence to treatment also had low adherence to vaccination , p 0.016), marital status (single, divorced or separated had lower adherence compared to married , p 0.015), physical exercise (patients who did not perform physical exercise had a lower adherence to those who do performing , p 0.063) and lack of information (p 0.085).
Conclusion: Adherence to vaccination is not appropriate in patients with IBD. Especific actions as optimizing the information provided to the patient about their illness and the need for adequate vaccination is the key to achieve better adhesion.

42 Metabolic syndrome in early rheumatoid arthritis and improvement after intensive treatment.

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Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Pacients with rheumatoid arthritis (RA) have an elevated risk of cardiovascular disease (CVD), predominantly ischaemic heart disease. Systemic inflammation is thought to be a key contributor to this risk and it is hoped early therapeutic suppression of inflammation will reduce cardiovascular (CV) events in the long term. The metabolic syndrome (MetS) is widely considered as an important risk factor for CVD. Data from 112 patients (69 % female , mean age 53 years, 55 % RF and 70 %anti-CCP positive) of Infliximab as Induction therapy for Early rheumatoid Arthritis (IDEA) multicenter doble blind randomized controlled trial study were analysed. Comparing week 78 to baseline there was a fall in the prevalence of MetS after intensive treatment according to the definition of World Health Organization (WHO) considering insulin resistance when HOMA-IR was within the highest quartile of HOMA-IR of the whole study population.

43 The microparticles induced by uremia transduce signals modulating oxidative stress and apoptosis in endothelial cells

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INTRODUCTION. The present state of chronic uremia microinflammation plays an important role in the development of endothelial damage in patients with chronic kidney disease (CKD). The vascular endothelium is essential for maintaining the integrity and overall homeostasis of blood vessels body. Before an injury and / or endothelial activation endothelial microparticles (MPE), which are small vesicles that participate as intercellular signaling elements occur.
OBJECTIVE. Assess ability MPE signaling induced uremia on cultured endothelial cells (HUVECs).
MATERIAL AND METHODS. To produce MPE, HUVECs were treated in culture with uremic serum (SU, 10%) and tumor necrosis factor α (TNF, 10 ng / ml) for 20 hours at 37 ° C and 5% CO2.
Supernatants were collected and ultracentrifuged 20.000g for 30 minutes. The microparticles of uremic serum (MPSU) and TNFα microparticles (MPTNFα) were used in cultures of HUVECs. Quantitated by flow cytometry Apoptosis (annexinV binding-AlexaFluor and propidium iodide) and oxidative stress (hydroethidine, which binds to reactive oxygen species, ROS).
RESULTS. Results are expressed as mean ± SD. Both MPTNFα and MPSU as occurred in increased oxidative stress (ROS) in relation to the control. In relation to apoptosis, MPTNFα and MPSU induced a significant increase compared to control cells. (See Table)
CONCLUSION. The MPE obtained by the activation of endothelial cells with TNFα and SU cause ROS and increased apoptosis in endothelial cells. In patients with CKD MPE (MPSU and MPTNFα) that might modulate cell internal processes such as apoptosis and oxidative stress; therefore these MPE can intervene in the possible development of endothelial dysfunction.

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