2º **Dia do Jovem Investigador**
do Instituto Nacional de Saúde Doutor Ricardo Jorge

08
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2017

Livro de resumos

REPÚBLICA PORTUGUESA
SAÚDE

SNS SERVIÇO NACIONAL DE SAÚDE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

2º Dia do Jovem Investigador

Instituto Nacional de Saúde Doutor Ricardo Jorge

Lisboa, 8 de maio de 2017

2º Dia do Jovem Investigador do Instituto Nacional de Saúde Doutor Ricardo Jorge

INSA – Lisboa
(auditório com vídeoconferência para o CSPGF)

08 maio 2017

Programa

09:00 **Receção de convidados**

09:30 **Abertura**

- **Fernando de Almeida,** Presidente do Conselho Diretivo
- **Manuela Caniça,** Presidente do Conselho Científico
- **Francisco George,** Diretor-Geral da Saúde
- **Fernanda Rollo,** Secretária de Estado da Ciência, Tecnologia e Ensino Superior

09:45 **Conferência convidada** (Moderadora: Manuela Caniça)

- Mechanisms for protein localization in bacterial cells. **Mariana Gomes de Pinho, ITQB** (beneficiária de uma ERC starting grant 2013/18)

10:30 **Stress response and adaptive evolution** (Moderador: João Paulo Gomes)

01: Gene-environment interactions in Autism Spectrum Disorders: excess copy number variants in genes regulating the effect of environmental exposure to toxicants. Apresentador: **João Xavier Santos**.

02: Genome-scale analysis of the non-cultivable *Treponema pallidum* reveals extensive within-patient genetic variation during syphilis. Apresentador: **Miguel Pinto**.

03: Within-patient evolution of blood-invading *Campylobacter jejuni* reveals bacterial genetic drivers of the pathogen-host "arms-race". Apresentador: **Vítor Borges**.

04: The effect of extreme cold temperatures on the risk of death in two major cities of Portugal. Apresentadora: **Liliana Antunes**.

11:30 Intervalo e visita aos posters **(P1 – P52)**

12:20 **Disease etiology and mechanisms** (Moderadora: Mafalda Bourbon)

05: Molecular regulation of the plasma membrane retention of disease-related chloride channels CFTR and NKCC2. Apresentadora: **Cláudia Loureiro**.

06: Stroma cells stimulate colorectal tumor cells to increase expression of tumor-promoting RAC1b. Apresentadora: **Joana Pereira**.

07: Lessons from colon cancer: How signal transduction pathways regulate alternative splicing of RAC1b. Apresentadora: **Vânia Gonçalves**.

08: Comparative breakpoint mapping and characterization of a *de novo* double translocation associated with a complex malformation phenotype. Apresentadora: **Mariana Marques**.

13:30 Almoço e visita aos posters **(P1 – P52)**

14:30 **Population-based epidemiology and biomonitoring** (Moderador: Baltazar Nunes)

09: Factors associated with psychological distress: Portuguese Health Examination Survey (INSEF) results. Apresentadora: **Ana Santos**.

010: Wildland firefighters and health effects. Apresentadora: **Ana Abreu**.

011: Geriatric study in Portugal on health effects of air quality in elderly care centers. Apresentadora: **Ana Mendes**.

012: Children exposure to multiple mycotoxins through food consumption: a holistic approach for risk assessment. Apresentador: **Ricardo Assunção**.

15:30 **Bridging health and technologies** (Moderador: João Paulo Teixeira)

013: Safety evaluation of novel polymeric nanocarriers for drug delivery using human osteoblasts. Apresentadora: **Kamila Dias**.

014: Splicing therapeutics for patients affected by lysosomal storage disorders. Apresentadora: **Liliana Matos**.

015: Activity-based probes as chemical tools for biomarker discovery ion chronic obstructive pulmonary disease by a proteomics-based approach. Apresentador: **Luís Carvalho**.

016: Influenza vaccine effectiveness in the Portuguese elderly during the 2016-17 season. Apresentadora: **Verónica Gomez**.

16:30 Intervalo e visita aos posters **(P1 – P52)**

17:30 **Encerramento e entrega dos prémios** (para a melhor comunicação oral e o melhor poster)

- **Rosalía Vargas,** Presidente da Agência Ciência Viva
- **Francisca Avillez,** Conselho Nacional de Ética para as Ciências da Vida
- **José Maria Albuquerque,** Vogal do Conselho Diretivo

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Conferência convidada

Mechanisms for protein localization in bacterial cells

Mariana Gomes de Pinho

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During infection, the host immune system interacts with the bacterial cell surface, a complex structure made of peptidoglycan, wall teichoic acids, lipoteichoic acids, capsule polysaccharide and peptidoglycan-attached proteins. A lot is known about the metabolic pathways for the synthesis of each individual component of the cell surface. Much less is known about the coordination between the synthesis of the peptidoglycan, the major structural component of the cell surface and the main inflammatory component of gram-positive bacteria, and the synthesis of the other molecules present at the surface. However, this coordination is essential for the construction of a surface capable of performing its biological functions in cell protection and morphology.

In our laboratory we investigate the temporal and spatial regulation of the enzymes responsible for the synthesis of the cell surface components, as well as their dependence on the underlying divisome. I will illustrate this research by describing the mechanisms of localization of the main enzymes involved in peptidoglycan synthesis – the Penicillin-Binding Proteins (PBPs).

Comunicações orais

O1

Gene-environment interactions in Autism Spectrum Disorder (ASD): excess copy number variants (CNV) in genes regulating the effect of environmental exposure to toxicants

João Xavier Santos[1,2,3]; Célia Rasga [1,2,3]; Muhammad Asif[1,2,3]; Ana Rita Marques,[1,2,3]; Astrid Moura Vicente[1,2,4]

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Background and objective(s): ASD is an heterogeneous neurodevelopmental disorder, with an estimated heritability of 50% and a complex genetic etiology. Recent studies implicate exposure to environmental factors in ASD. We thus hypothesize that ASD is the result of the interaction of a genetic susceptibility with exposure to environmental toxicants early in development. Here we tested whether ASD individuals have an excess of CNVs targeting genes involved in control of toxicant effects, including detoxification or regulation of barrier permeability (blood-brain barrier, placenta and respiratory cilia).

Materials and methods: CNVs targeting detoxification and barrier genes were analyzed in 2446 children with ASD from the *Autism Genome Project*. Deletions and duplications were compared with control individuals from the *Database of Genomic Variants* (N=10355), and inheritance status was assessed.

Results: Through literature and database review we identified 519 relevant detoxification and barrier genes. CNVs targeted 186 (35,8%) of these genes in 1163 (47,1%) ASD subjects from the AGP dataset. 31 (16,7%) of these genes were exclusively targeted by CNVs in ASD patients, while 24 (18,3%) genes were more frequently targeted by rare CNVs (present in less than 1% of control population) in ASD patients compared to controls, after Bonferroni correction ($P<3,2x10^{-4}$). Among these genes are UGT, CYP, GST and ABC gene families, known to interact with environmental toxicants associated with ASD, such as bisphenol A, polycyclic aromatic hydrocarbons, pesticides and phthalates.

Discussion and conclusion(s): This study reinforces the hypothesis that the exposure to environmental factors in genetically susceptible individuals contributes to ASD risk.

Acknowledgements: JXS is a fellow of the *BioSys-PhD programme* and a recipient of a scholarship funded by *Fundação para a Ciência e Tecnologia* (FCT) (PD/BD/114386/2016). ASDEU (Autism Spectrum Disorders in the European Union)

O2

Genome-scale analysis of the non-cultivable *Treponema pallidum* reveals extensive within-patient genetic variation during syphilis

M. Pinto[1,2], V. Borges[1,2], M. Antelo[1], M. Pinheiro[3], A. Nunes[1,2], J. Azevedo[4], M.J. Borrego[1], J. Mendonça[5], D. Carpinteiro[5], L. Vieira[5] J.P. Gomes[1,2]

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Insights into the genomic adaptive traits of *Treponema pallidum*, the causative bacterium of the sexually transmited disease syphilis, have long been hampered by the absence of *in vitro* culture models and its obligatory propagation in rabbits. Prior to this work, *T. pallidum* was one of the least sequenced bacterial pathogens, with only six genomes obtained. As they derived from rabbit inoculations, concerns are raised that these might not reflect what occurs during human infection due to host-specific adaptation. Here, through a targeted strategy never applied to uncultivable bacterial human pathogens, we have bypassed the culture bottleneck to directly study, for the first time, *T. pallidum* whole-genome during human infection. While studying 25 syphilitic patients, we observed a scenario of discreet *T. pallidum* interstrain single nucleotide polymorphism-based microevolution, contrasting with a rampant within-patient genetic heterogeneity mainly targeting multiple phase-variable loci and a major antigen-coding gene (*tprK*). TprK demonstrated remarkable variability and redundancy, intra- and interpatient, suggesting ongoing parallel adaptive diversification during human infection. The small overlap observed between human- and rabbit-derived TprK antigenic peptides points that host-specific pressures ultimately determine its epitope repertoire. Finally, patient-derived genomes possess novel mutations targeting a penicillin-binding protein, unveiling it as a candidate target to investigate the impact on penicillin susceptibility (the current recommended treatment). Our findings, while vastly increasing the available *T. pallidum* genomic information, decode the major genetic mechanisms by which the bacteria promotes immune evasion and survival, and demonstrate the exceptional power of characterizing the changes that the pathogen undergoes during human infection.

Acknowledgements: This study was partially supported by grant EXPL/BIA-MIC/0309/2013 from the Fundação para a Ciência e a Tecnologia (FCT).

O3

Within-patient evolution of blood-invading *Campylobacter jejuni* reveals bacterial genetic drivers of the pathogen-host "arms-race"

Vítor Borges[1], Marta Ferreira[1], Andrea Santos[2], Luís Vieira[3],João Paulo Gomes[1], Mónica Oleastro[2]

[1]Bioinformatics Unit, National Institute of Health, Lisbon, Portugal; [2]National Reference Laboratory for Gastrointestinal Infections, National Institute of Health, Lisbon, Portugal; [3]Innovation and Technology Unit, National Institute of Health, Lisbon, Portugal.

Campylobacter jejuni is a major cause of gastroenteritis worldwide, with fluoroquinolone-resistant *C. jejuni* being one of the WHO priority pathogens for new antibiotics development. We aimed to understand how this pathogen shapes its genome towards invasion and persistence in human blood. Thus, we studied the genome of an invasive *C. jejuni* strain during translocation across the intestinal tract and further blood persistence during ~1 month. We performed whole-genome sequencing and phenotypic analyses on same-patient *C. jejuni* isolates collected from stool and blood. We observed a within-patient evolutionary scenario of the pathogen strongly marked by genomic changes driving invasion and adaptation to the human blood environment, including: i) the acquisition of protein-changing mutations affecting bacterial motility and chemotaxis; ii) one mutation affecting a surface-exposed domain of a *C. jejuni* major antigen; iii) the emergence of a mutation targeting a gene (*oorC*) associated with *C. jejuni* response to oxygen availability; and, iv) the activation of the invasion protein CipA. Notably, all same-patient isolates showed to be resistant to ciprofloxacin, but the end-point blood-evolving isolate revealed an extended spectrum of resistance to fluoroquinolones. This extended resistant profile could be linked both to the Asp90Gly mutation affecting DNA gyrase subunit A (never described in *C. jejuni*) and to the fact that the patient reported ciprofloxacin therapy during the infection timeframe. While this study highlights *C. jejuni* genetic drivers of blood invasion and persistence, it further provides unprecedented information about how *C. jejuni* population change upon blood invasion and how resistant clones emerge during infection.

O4

The effect of extreme cold temperatures on the risk of death in two major cities of Portugal

Liliana Antunes [1,2], Susana Silva[2], Jorge Marques [1], Baltazar Nunes [2], Carlos Dias [2], Sílvia Antunes [1]

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Periods of extreme cold have been shown to be associated with an increase risk of death during the winter, in particular with cardiovascular and respiratory deaths. Despite the temperate climate, Portugal presents one of the highest excess winter deaths in Europe. The objective of the present work was to estimate the effect of minimum temperature in Lisbon and Porto aiming the development of a real-time warning system.

Poisson regression models combined with distributed lag non-linear models were applied to assess the exposure-response relationship and lag patterns of the association between minimum temperature and all cause and both respiratory and circulatory system diseases mortality from 1992 to 2012, for the period of November to March, in Lisbon and Porto. Models were adjusted for the confounding effect of influenza, for over dispersion and population size.

Minimum temperature effect was higher for respiratory and circulatory system diseases mortality than for all cause mortality. The overall Relative Risk (RR) for respiratory and circulatory mortality was 2.34 [IC 95: 2.13 – 2.57] in Lisbon and 1.98 [IC 95: 1.79 – 2.20] in Porto. For all cause mortality was 1.83 (CI 95% [1.72 – 1.95]) in Lisbon and 1.67 (CI 95% [1.57 – 1.79]) in Porto. Overall, cold effect persists from 1 to a maximum of 26 to 28 days and the maximum risk was estimated after 6-7 days.

The results found in this work were also observed in other European cities, namely the stronger and more persistence through time association of minimum apparent temperature with respiratory system disease mortality.

Acknowledgements: This work was financially supported by national funds from FCT – FRIESA PROJECT (EXPL/DTP-SAP/1373/2013).

O5

Molecular regulation of the plasma membrane retention of disease-related chloride channels CFTR and NKCC2

Cláudia Loureiro[1,2], Paulo Matos[1,2], Luka Clarke [2] and Peter Jordan [1,2]

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Three disease-related chloride transport proteins, CFTR (in cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD)), NKCC2 and KCC3 (in kidney function and hypertension), were found to be specific targets for protein kinase Syk which phosphorylates a specific tyrosine residue in each channel. Tyrosine phosphorylation downregulates the amount of CFTR [1] present at the plasma membrane and a better understanding of this process may reveal novel therapeutic options for CF patients. Thus, we determined the cellular adaptor proteins able to recognize the phospho-tyrosine modification and mediate traffic to the plasma membrane.

For adaptor protein identification, we used biotinylated synthetic peptides containing the respective CFTR, NKCC2 or KCC3 phospho-tyrosines as baits and isolated adaptor proteins from physiologically relevant human or mouse cell lysates. After the peptide pull-down the samples were sent for mass spectrometry by Nano-LC-Triple TOF analysis to identify the obtained complex mixture of proteins. Following a bioinformatics analysis the best candidates were chosen for experimental validation and we identified the adaptor protein SHC1 that binds specifically to the channel peptides only in their phospho-tyrosine form. We are currently investigating the interaction between CFTR and SHC1 and its impact on CFTR trafficking and plasma membrane anchoring. The results are expected to reveal the molecular mechanism underlying tyrosine-phosphorylation of these chloride channels and may suggest novel therapeutic targets for diseases like CF and hypertension.

Acknowledgments: Funding support by FCT (PD/BD/52488/2014 and centre grant UID/MULTI/04046/2013 to BioISI).

Reference: [1] Mendes et al., *Mol Cell.Biol.* **2011***, 31,* 4076-4086.

O6

Stroma cells stimulate colorectal tumor cells to increase expression of tumor-promoting RAC1b

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Background and objectives: An inflammatory microenvironment is a tumor-promoting condition that provides survival signals to which cancer cells respond with changes in their gene expression. One key gene regulatory mechanism that responds to extracellular signals is alternative splicing. For example RAC1B, a RAC1 alternative splicing variant that we previously identified in a subset of BRAF-mutated colorectal tumours, was found increased in samples from inflammatory bowel disease patients or following experimentally-induced acute colitis in a mouse model. The main goal of this work is to determine the pro-inflammatory signals that lead to increased RAC1b expression in colorectal cells.

Materials and methods: Caco-2 colorectal cells were either grown as polarized cell monolayer on porous filter membranes and then co-cultured with different stromal cell lines (fibroblasts, monocytes and macrophages) for 48 h, or grown as cysts in 3D matrices. RAC1B expression was analysed by RT-PCR, Western blot and confocal fluorescence microscopy.

Results and discussion: Culture conditions for polarized 2D and 3D models were established as physiologically more relevant colon cell models. Co-culture experiments with polarized cells revealed that the presence of fibroblasts or M1 macrophages induced a transient increase in RAC1B protein levels in the colorectal cells, accompanied by a progressive loss of epithelial organization. The cytokines secreted by fibroblasts and macrophages are currently being identified

Conclusions: Our data indicate that extracellular signals from stromal cells can affect gene expression in colorectal cancer cells. The observed increase in alternatively spliced RAC1B will help to understand the tumor-promoting effect of inflammation and identify novel therapeutic targets.

Acknowledgements: Funding Suport: fellowship BRJ-DGH 2012_oncologia from INSA; grant UID/MULTI/04046/2013 *and SFRH/BD/109162/2015 from FCT*.

O7

Lessons from Colon Cancer: How Signal Transduction Pathways Regulate Alternative Splicing of RAC1b

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In colon cancer distinct genetic subtypes have been described, one of which involves overexpression of RAC1b, a variant generated by alternative splicing. Aberrant splicing is known to occur in cancer and can be caused by mutation in a gene or splicing factor but also represent a dynamic response to oncogene-induced cellular signaling and in this case it may be pharmacologically targeted. Here we explore how signaling pathways are involved in the deregulation of alternative RAC1b splicing in tumor cells.

HT29 colorectal cells represent serrated colorectal tumors with *BRAF* gene mutation V600E in one allele and RAC1b overexpression. Cells were transfected with shRNA vectors directed against target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24h later by Western Blot and qRT-PCR. Treatment with kinase inhibitors or anti-inflammatory drugs was performed 24h prior to cell lysis.

Because cell signaling involves protein kinases that can be targeted by inhibitory drugs, 20 candidate splicing-regulatory protein kinase genes were depleted by RNAi in HT29 cells. Two kinases, SRPK1 and GSK3β, were found required to sustain RAC1b levels and both were shown to act upon the phosphorylation of splicing factor SRSF1, which binds to and promotes the inclusion of the alternative exon in RAC1b. Reduced SRSF1 phosphorylation decreased its nuclear translocation and concomitantly RAC1b splicing. This regulatory pathway is controlled by GSK3β and was found to be inhibited specifically by the anti-inflammatory drug ibuprofen.

Together, our results demonstrate that oncogenic signal transduction pathways deregulate alternative splicing and this may be drug revertable.

Acknowledgements: This work was supported by the Fundação para a Ciência e Tecnologia, Portugal and Association Maratona da Saúde.

O8

Comparative breakpoint mapping and characterization of a *de novo* double translocation associated with a complex malformation phenotype

Marques M[1], Freixo J[2], Gonçalves RM[2], Talkowski ME[3], Morton CC[4], David D[1]

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Background and objective(s): Causal relationship between congenital anomalies and associated balanced chromosomal rearrangements are expected to occur in up to 40% of the affected subjects. In this study, we aim comparative mapping of the breakpoints and identification of candidate genes responsible for the malformation phenotype characterized by intrauterine growth retardation, developmental delay, brain malformations and refractory epilepsy reported in a subject with an apparently balanced *de novo* double chromosomal translocation - t(2;7)(q23;q32),t(5;6)(q23;q26)dn.

Materials and methods: Unbalanced genomic alterations were screened by whole-genome array. Translocation breakpoints were comparatively mapped by array painting with genomic amplicons of the derivative chromosomes and by large-insert whole genome sequencing (liWGS). Subsequently, junction fragments were amplified and sequenced by Sanger sequencing.

Results: Both array painting and liWGS identified t(2;7)(q23.3;q32.1),t(5;6)(q23.2;q26)dn breakpoints. However, only liWGS unraveled an additional 48kb cryptic excision/insertion and a 1.5Mb inversion of the 5q23.2 sequence adjacent to the translocation breakpoint on der(6).

The breakpoints of t(2;7)(q23.3;q32.1) disrupt *PRPF40A* at IVS5 and *SND1* at IVS16, respectively. Regarding t(5;6)(q23.2;q26), the 6q26 breakpoint disrupts *PACRG* at IVS3. The excised 48kb fragment at 6q22.33, containing *PTPRK* exon 7, is inserted 36Mb further distal at 6q26.

Disruption of *PRPF40A* and *PACRG,* in association with misregulation of *CACNB4*, *RBM28, PARK2*, *QKI* and *LAMA2* located within the breakpoints' topological association domains are the suggested candidate genes responsible for this complex malformation phenotype.

Discussion and conclusion(s): Causal relationship between the reported phenotype and observed genomic alterations was unveiled. Furthermore, comparative analysis of this complex structural chromosomal rearrangement confirmed that only liWGS is able to identify the full spectrum of genomic structural alterations.

Acknowledgements: This study was supported by FCT project *HMSP-ICT/0016/2013*.

O9

Factors associated with psychological distress: Portuguese Health Examination Survey (INSEF) results

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Background and objective(s): Psychological distress is a multi-factorial construct that relates to poor psychological function and worse quality of life. This study examines the association between psychological distress and sociodemographic, health status and social support variables.

Materials and methods: We used data from the First National Health Examination Survey (INSEF) conducted in Portugal in 2015. Psychological distress was assessed on a probabilistic sample of 4911 individuals aged 25-74 years old through the Mental Health Inventory 5. Poisson regression was used to estimate adjusted prevalence ratios (aPR) of psychological distress according to sex, age group, marital status, cohabiting, education, employment status, co morbidities, functional status, perceived health status and social support. It combines blood pressure measurement, blood collection and interview.

Results: Psychological distress was reported by 22.5% [95%CI: 20.7, 24.5] of interviewed individuals. Women (30.5%, aPR=1.97 [95%CI 1.7-2.46]), widows (46.2%, aPR=1.42 [1.1-1.8]) and unemployed (28.6%, aPR=1.26 [1.0-1.5]) individuals were more likely to report psychological distress. Prevalence of psychological distress was higher among individuals with at least one chronic disease (28.1%, aPR=1.57 [95%CI 1.96-1.8]) and those perceiving their health as bad or very bad (57.5%, aPR=3.02 [95%CI 2.5-3.6]). Low (35.2%, aPR=1.47 [1.2-1.8]) or moderate (24.5%, aPR=1.24 [1.1-1.3]) perceived social support also increased psychological distress prevalence.

Discussion and conclusion(s): In Portugal psychological distress affects 225 per 1000 adults aged 25-74 years old. INSEF results suggest that multiple factors across different domains have a bearing on psychological well-being. Specific population groups are probably more at-risk of developing mental health problems: women, unemployed and widows.

Acknowledgements: EEA Grants

O10

Wildland firefighters and health effects

Ana Abreu[1,2], Armanda Teixeira-Gomes[1,2], Susana Silva[1], Simone Morais[3], Maria do Carmo Pereira[4], Adília Fernandes[5], João Paulo Teixeira[1,2], Solange Costa[1,2]

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Portugal is a high risk country for forest fires supported by a human force of 30,000 firefighters. Firefighters are often exposed to many toxic combustion products, including known carcinogens. Genotoxicity evaluation is a valuable tool for studying the most important occupational hazards allowing a reasonable epidemiological evaluation of cancer prediction. The aim of this study was to evaluate DNA damage in Portuguese wildland firefighters when compared with the general population.

A total of 123 individuals were engaged in the study, 60 volunteer firefighters and 63 non-exposed control subjects. Total and oxidative DNA damage were evaluated by comet assay in whole blood samples; oxidative damage was measured by formamidopyrimidine glycosylase (FPG).

Both total and oxidative damage were increased in firefighters compared to controls. However, only total DNA damage was significantly higher. The influence of life style factors and work-related variables (duration and recent exposure) was also studied, but no significant effect was found.

To our knowledge, this is the first Portuguese study to investigate the potential genotoxic effects of wildland firefighting exposure. Results provide new data regarding potential mechanisms underlying the health effects of wildland firefighting exposure. Hence this study may offer the support needed to implement effective measures in order to protect firefighter's health, including regular monitoring and surveillance activities, such as medical surveillance, good practice campaigns, training programs and implementation of written policies and procedures.

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O11

Geriatric Study in Portugal on Health Effects of Air Quality in Elderly Care Centers

Ana Mendes[1,2], Amália Botelho[3], Nuno Neuparth[3], Iolanda Caires[3], Teresa Palmeiro[3], Ana Luísa Papoila[4,5], Pedro Martins[3,5], Paulo Paixão[3], Joana Belo[3,6], Daniel Aelenei[7], João Viegas[8], Susana Azevedo[7], Lívia Aguiar[1,2], Paula Neves[1], Manuela Cano[1], Fátima Aguiar[1], João Paulo Teixeira[1,2]

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The GERIA Project provides valuable information on the main characteristics of indoor air, its pollutants and buildings. This study took place in two main Portuguese cities, Lisbon and Porto. Within its 1st phase, 931 residents from 53 elderly care centers (ECC) were studied. The 2nd phase completed a thorough analysis based on the preliminary phase study, involving 817 residents from 18 ECC in Lisbon.

Thermal comfort, levels of carbon dioxide, microbiological agents and particulate matter where somewhat unacceptable in 20 to 35% of the ECC rooms studied. Although most of the ECC had satisfactory ventilation rates a significant number revealed low ventilation rates. In general the ventilation is not provided by properly designed systems, but is due to the high air permeability of the envelope (windows and doors) generating a poor indoor comfort.

Health perception was low, particularly for those with respiratory diseases, but most of the residents had a favorable perception of their overall quality of life, which included ECC environmental conditions. The most reported respiratory diseases were allergic rhinitis and asthma, presenting frequent cough and wheezing in the previous 12 months. It was shown a relationship between bedroom ventilation during the night, and the presence of respiratory symptoms, respiratory diseases and lung function defects. The microbiological characterization of acute respiratory episodes was positive in two thirds of all cases and detected great diversity of agents.

It is very important to revise overcrowding, change inadequate ventilation, identify sources of contamination, control thermal parameters and adjust clothing to environmental characteristics.

Acknowledgements: This project have been awarded with the Arnaldo Sampaio 2016 Award as better Public health research work carried out in Portugal. This work was supported by GERIA Project (www.geria.web node.com): PTDC/SAU-SAP/116563/2010 and a PhD Grant 585(SFRH/BD/72399/2010) from Fundação para a Ciência e Tecnologia through Operational Competitiveness Programme as part of QREN. Authors are indebted to all participants in the GERIA Project as well as to the professionals and elderly residents in the ECC and the authorities from which they depend.

O12

Children Exposure to Multiple Mycotoxins through Food Consumption: A Holistic Approach for Risk Assessment

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Background and objective(s): Humans can be exposed to multiple chemicals at once from a variety of sources, including food. Human risk assessment of multiple chemicals poses several challenges to scientists, risk assessors and risk managers. A lack of data regarding multiple mycotoxins children risk assessment is a reality and a deep effort should be set in motion to overcome this gap. The present work, developed within the MYCOMIX project, aimed to assess the risk associated to the exposure of Portuguese children (< 3 years old) to multiple mycotoxins through consumption of foods primarily marketed for this age group.

Materials and methods: A holistic approach was developed applying deterministic and probabilistic tools to calculate 13 mycotoxins daily intake values, integrating children food consumption (3-days food diary), mycotoxins occurrence (HPLC-UV, HPLC-FD, LC-MS/MS and GC-MS), bioaccessibility (standardized *in vitro* digestion model) and toxicological data (*in vitro* evaluation of cytotoxicity, genotoxicity and intestinal impact).

Results: Contrary to others, aflatoxins exposure suggested a potential health concern for the high percentiles of intake. In addition, patulin and ochratoxin A suggested a combined toxic effect with synergism at low doses and antagonism at higher doses. These results require a special attention due to the potential loss of intestinal membrane integrity and the development of intestinal diseases.

Discussion and conclusion(s): The present original results contribute to improve the risk assessment of multiple mycotoxins, particularly associated to children exposure, and thereby are expected to constitute a considerable contribution to protect the children health.

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O13

Safety Evaluation of Novel Polymeric Nanocarriers for Drug Delivery Using Human Osteoblasts

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The development of novel nanocarriers has been recognized as a promising approach to improve drug release profiles on targeted sites, being the assessment of their biocompatibility and safety a critical point of the process. The objective of this work was to characterize the cellular interactions and the potential toxicity of the novel developed polymeric nanoparticles (NPs), using human osteoblasts.

For this purpose, Poly(methyl methacrylate) (PMMA) and PMMA-Eudragit RL 100 (PMMA-Eud, 50:50) NPs were produced and their physicochemical properties were characterized. The safety evaluation of both NPs was conducted through the characterization of cellular uptake (fluorescence microscopy), cyto- and genotoxicity (MTT, Comet and Micronucleus assays) using both normal and differentiated osteoblast cell line (MG-63-ATCC®CRL-1427™).

The results confirmed the successful uptake of PMMA and PMMA-Eud by the cells. Both NPs were not cytotoxic in differentiated cells, although a moderate toxicity was detected in undifferentiated cells. As to their genotoxic potential, NPs induced primary DNA damage in osteoblasts, especially under a short-term exposure. Noteworthy, none of the NPs caused chromosome breaks, indicating that the primary DNA lesions were not converted into permanent genetic damage. On the other hand, an increased cell proliferative capacity was noted for PMMA-NPs, which deserves further investigation.

In conclusion, the safety assessment of the two NPs indicated that both are biocompatible but display a weak genotoxicity that should be explored in other *in vitro* endpoints, e.g., gene mutation, and *in vivo* studies. Moreover, understanding how physicochemical features relate to toxicity will support the design of safer formulations for biomedical purposes.

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O14

Splicing therapeutics for patients affected by lysosomal storage disorders

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Splicing defects are among the most frequent pathogenic mechanisms underlying genetic diseases. Thus, the development of therapeutic strategies targeting RNA represents an important opportunity to correct faulty splicing, opening the prospects of treatment for numerous genetic disorders. The vast majority of RNA-based approaches have exploited, *in vitro* and *in vivo*, the use of antisense oligonucleotides or modified U1 snRNAs to overcome different splicing mutations.

Lysosomal storage disorders (LSDs) are a group of inherited diseases that can result in severe and progressive pathology due to a specific lysosomal dysfunction. In several patients, splicing mutations have been identified and are frequently associated with particular types of LSDs worldwide. Some treatment strategies are already available for conventional LSDs, but yet with some limitations. Therefore, for splicing mutations, splicing therapeutics might represent a crucial option or an important adjunct of other treatments.

In this study, we have used a modified U1 snRNA that completely matches the splice donor site of *HGSNAT* gene exon 2, which corrected the effect of the common 5' splice site mutation c.234+1G>A in Mucopolysaccharidosis IIIC (1). In another approach using an antisense oligonucleotide (AO) we have succeeded in the correction of the c.66G>A splicing mutation in *CSTB* gene (Unverricht–Lundborg disease). Besides that, we have performed the functional analysis of some *IDS* gene splicing mutations (Mucopolysaccharidosis II) and used AOs to exploit an alternative therapy for one of those mutations (c.1122C>T on exon 8) (2).

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O15

ACTIVITY-BASED PROBES AS CHEMICAL TOOLS FOR BIOMARKER DISCOVERY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE BY A PROTEOMICS-BASED APPROACH

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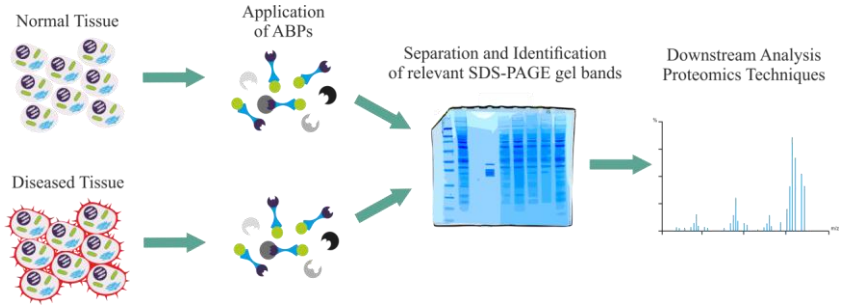
Chronic Obstructive Pulmonary Disease (COPD) is characterized by lung inflammation, progressive weakening of its structure and irreversible narrowing of the airways. The World Health Organization predicts it will become the third cause of death worldwide in 2030[1]. Biomarkers to diagnose and predict COPD progression are urgently needed. The central hypothesis of this project is that COPD inflammation modulates proteolytic enzymes in the lungs, like Human Neutrophil Elastase (HNE), which contribute to the pathology development and are clinically relevant for its diagnosis and prognosis. Proteolytic activity can be measured by activity-based probes (ABPs), which target only active forms of enzymes, providing dynamic profiles of enzyme activities in biological contexts[2]. In this work, we developed a multidisciplinary approach combining medicinal chemistry and proteomics to generate new COPD diagnostic tools. Following our extensive work with HNE inhibition[3], we developed a library of HNE inhibitors and ABPs that target HNE based on the 3-oxo-β-sultam[4] warhead, using copper-assisted azide-alkyne Huisgen cycloaddition[5]. Our inhibitors revealed outstanding potency against HNE, with inhibition values in the nanomolar region. ABPs are currently being tested for their ability to target HNE in complex proteomes by gel-based assays and MALDI-TOF analysis. Once validated, our probes will provide protein activity quantification on patient derived biospecimens in a proteomics-based approach (Scheme 1). We envisage the study and validation of HNE as a potential biomarker in COPD. The outcome of this project will be a breakthrough in the field and ultimately lead to important advances for diagnostic tool development and biomarker discovery in COPD.



Scheme 1: Proteomics-based approach in the discovery and validation of biomarkers using activity-based probes.

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O16

Influenza Vaccine Effectiveness (IVE) in the Portuguese elderly during the 2016-17 season

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Background and objective(s): This study aims to provide estimates of IVE against confirmed medically attended influenza and confirmed hospitalized influenza in the Portuguese aged 65+, during the 2016-17 season.

Materials and methods: We used the test negative design in both settings. On Primary Care (PC) setting, laboratory confirmed medically attended influenza patients were compared to influenza negative patients, whereas at influenza positive severe acute respiratory infection (SARI) patients were compared to negative ones. Epidemiological data was collected via questionnaire and the influenza diagnosis in nasopharyngeal swabs by RT-PCR. IVE was estimated as 1-OR of being vaccinated in cases vs controls, adjusted for age, ≥2 underlying conditions and time trough non-conditional logistic regression.

Results: In both settings, *influenza* subtype A(H3N2) was detected in all influenza positive patients. Between weeks 46/2016 and 8/2017, in PC setting, 61 patients were recruited, of which 33% were influenza cases and 67% controls. The vaccine coverage (VC) was 50% in cases and 47% in controls, which corresponds to an adjusted IVE of 11% (CI95%: -238%:63%). In hospital setting, 85 SARI patients were recruited, 54% were cases and 46% controls. The VC was 31% in cases and 50% in controls, with adjusted IVE of 49% (CI95%: -55.4%:83.1%).

Discussion and conclusion(s): The adjusted IVE was 11% in the PC setting and 49% in HL, indicating low protection of the seasonal 2016-7 vaccine in the elderly population. The small sample size should be considered in the interpretation, however it should be noted1 that these estimates are according to results from other studies.

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Posters

P1

Inducing a new start

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The lack of good disease models limits the understanding of the human pathophysiologic mechanisms and hinders investigation reserch and development of new therapies. In 2006, Yamanaka's group expressed four transcription factors (Oct4, Sox2, Klf4, and c-Myc) producing induced pluripotent stem cells (iPSCs), allowing the development of new strategies for pathogenesis modeling and drug testing. iPSCs generated from somatic cells from patients are a desirable source for patient-specific studies since they maintain the patient's genetic background.

In this work we ultimately aim to develop induced pluripotent stem cells (iPSCs) from Lysosomal storage disorders (LSDs) patient's fibroblasts and normal controls to produce disease models. Thus, using iPSCs methods to generate the cell-targets to reproduce the disease may create an ideal model for studying pathogenic mechanisms. The initial biological material being used consists of commercially obtained human control dermal fibroblasts. This type of material guarantees better consistency in technical conditions. We are testing two different non-integrative polycistronic plasmid vectors in order to achieve forced expression of the Yamanaka's transcription factors. For this achievement, transformation conditions with different vehicles of delivery were tested: different transfection reagents, concentration ratios and timings were compared.

Since we are beginning this work from zero, only few very preliminary results were obtained and will be presented.

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P2

Search for new modulators of F508del-CFTR retention at the plasma membrane of epithelial cells

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Cystic Fibrosis (CF) is a devastating genetic disease that is caused by mutations in CFTR (CF transmembrane conductance regulator), a chloride (Cl−) channel normally expressed at the surface of epithelial cells. The most frequent mutation, the deletion of a phenylalanine residue at position 508 (F508del), causes the protein to misfold and be prematurely degraded. A few compounds were identified that showed promise in overcoming the folding and trafficking defects of F508del-CFTR. However these compounds produced only marginal effects in clinical trials. The host laboratory showed that these effects could be enhanced by stimulating signalling pathways that tether the rescued channel to the actin cytoskeleton. Based on this knowledge we are currently using a proteomic approach to identify novel key interactors of the pharmacologically rescued F508del-CFTR at the plasma membrane (PM). Our preliminary results show specific protein-protein interactions in the rescued F508del-CFTR complex compared with normal-CFTR. We will now validate functionally and biochemically these significant hits and access the effectiveness of their manipulation in bronchial epithelial cellular cultures to enhance the efficacy of F508del-CFTR restoration by the available pharmacological correctors.

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P3

Suppression therapy as a novel approach for genetic diseases and cancer

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Premature translation-termination codons (PTCs or nonsense codons) can arise from mutations in germ or somatic cells. The introduction of a PTC into an mRNA can trigger nonsense-mediated decay (NMD), an important mRNA surveillance mechanism that typically recognizes and degrades mRNAs containing PTCs to prevent the synthesis of C-terminally truncated proteins potentially toxic for the cell. The physiological importance of NMD is manifested by the fact that about one third of genetic disease-associated mutations generate PTCs, including β-thalassemia.

In recent years, a novel therapeutic approach entitled suppression therapy has been developed based on low molecular weight compounds to induce the translation machinery to recode a PTC into a sense codon, the so called "readthrough". Here, by using a model of constructs containing the firefly luciferase gene as a reporter gene for β-globin transcripts that result from PTCs, we intend to prove the principle that the suppression therapy can restore enough β-globin protein to outweight the manifestations of β-thalassemia. Our preliminary results show that both the aminoglycoside G418 and non-aminoglycoside PTC124 do not seem to be able to suppress the nonsense mutation at codon 26 or 39 of the human β-globin mRNA in cultured HeLa cells, as reflected on the firefly luciferase activity and protein levels assessed by bioluminescence assays and Western blot, respectively. Regarding future directions, a deeper study on the use of G418 and PTC124 as efficient suppression agents for PTC-associated diseases treatment will be performed as it offers a major potential to treat a wide range of inherited pathologies.

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P4

LDLR, *APOB* and *PCSK9* variants associated with Familial Hypercholesterolaemia – application of ACMG guidelines for variant interpretation

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Background and objective(s): Familial hypercholesterolaemia (FH) is an autosomal disorder of lipid metabolism presenting with increased cardiovascular risk. Although more than 1700 variants have been associated with FH, the great majority have not been proved functionally to affect the LDL receptor cycle.

Materials and methods: We aimed to classify, following American College of Medical Genetics and Genomics (ACMG) guidelines, all described variants associated with FH in different databases and individual reports to establish the proportion of variants that lack evidence to support their pathogenicity. A worldwide overview of FH variants has also been performed.

Results: A total of 2104 unique variants were found associated with FH but only 166 variants have been proven by complete in vitro functional studies to be causative of disease. Additionally, applying the ACMG guidelines, 1097 variants were considered pathogenic or likely pathogenic. Only 7 variants were found in all 5 continents.

Discussion and conclusion(s): The lack of functional evidence for about 85% of all variants found in FH patients can compromise FH diagnosis and patient prognosis. ACMG classification improves variant interpretation but functional studies are necessary to understand the effect of about 40% of all variants reported. Nevertheless ACMG guidelines need to be adapted to FH for a better patient diagnosis.

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P5

Analysis of translation of 5' untranslated regions in colorectal cancer

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Carcinogenesis is characterized by a continuous accumulation of genetic alterations that changes the overall gene expression profiles. Those alterations have been studied by microarray and RNA sequencing that measure the abundance of mRNA but do not provide information on protein synthesis, a step closer to the end-point of gene expression. Ribosome profiling (Ribo-seq) emerges to monitor *in vivo* translation by deep sequencing of ribosome-protected mRNA fragments. This technique detects ribosomes outside of known protein-coding regions, identifying translation of upstream open reading frames (uORFs) within 5' untranslated regions (5'UTRs). Our aim is to determine the role of specific uORFs in cancer tumorigenesis, mainly in colorectal cancer (CRC). Thus, we will use already available Ribo-seq data from different cancer cell lines to get the 5'UTR translation profiles to choose potential uORFs-containing targets. Then, we will analyze the role of such uORFs in translational regulation and study the biological function of those translatable uORFs at the level of cell viability and proliferation, and acquisition of malignant features to understand their involvement in CRC development. Based in 5'UTR ribosome occupancy profiles from Ribo-seq analysis we chose ABCE1, PAIP2, eIF4G2 and eIF2A as our uORFs-containing mRNAs. By semi-quantitative RT-PCR ABCE1 transcript is shown down- and up-regulated in HCT116 and SW480 cells, respectively, in comparison to the non-neoplasic colorectal cell line (NCM460). We are now mapping the exact 5'-end of each transcript 5'UTR by circular rapid amplification of cDNA ends to finally clone them in a reporter plasmid and study their function in translational control.

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P6

Alpha-thalassemia due to novel deletions and complex rearrangements in the subtelomeric region of chromosome 16p

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Introduction: Inherited deletions removing the α-globin genes and/or their upstream regulatory elements (MCSs) give rise to alpha-thalassemia, one of the most common genetic recessive disorders worldwide. The pathology is characterized by microcytic hypochromic anemia due to reduction of the α-globin chain synthesis, which are essential for hemoglobin tetramerization.

Material and Methods: In order to clarify the suggestive α-thalassemia phenotype in ten patients, we performed Multiplex Ligation-dependent Probe Amplification with commercial and synthetic engineered probes, gap-PCR, and Sanger sequencing to search for deletions in the subtelomeric region of chromosome 16p.

Results: We have identified five distinct large deletions, two of them novel, and one indel. The deletions range from approximately 3.3 to 323 kb, and i) remove the whole α-globin cluster; or ii) remove exclusively the upstream regulatory elements leaving the α-globin genes structurally intact. The indel consists in the loss of MCS-R2 (HS-40), which is the most important distal regulatory element for the α-globin gene expression, and the insertion of 39 bp, seemingly resulting from a complex rearrangement involving two DNA segments (probably from chromosome 3q) bridging the deletion breakpoints with a CC-bp orphan sequence in between. Finally, in one patient no α-globin deletion or point mutation were found. This patient revealed to be a very unusual case of acquired alpha-thalassemia associated with a myelodysplastic syndrome.

Conclusions: Our study widens the spectrum of molecular lesions by which α-thalassemia may occur and emphasizes the importance of diagnosing large α-zero-deletions to provide patients with appropriate genetic counseling.

P7

Translational control of the human erythropoietin expression *via* an upstream open reading frame in cardiac tissue

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Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific translation. Translational regulation of specific transcripts mostly occurs at mRNA translation initiation and is mediated *via* different *cis*-acting elements present in the mRNA 5' untranslated region (5'UTR), such as upstream open reading frames (uORFs). uORFs modulate translation of the main ORF decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF.

Human erythropoietin (EPO) is a glycoprotein synthesized and released mainly from the kidney, which has a key role in hematopoiesis. However, recent studies have revealed that EPO is a multifunctional molecule produced and utilized by many tissues that rapidly responds to different cell stress stimuli and tissue injuries. The 5'UTR sequence of the human EPO mRNA has one uORF with 14 codons, which is conserved among different species, indicating its potential role in translational regulation.

To test whether EPO expression is translationally regulated in response to ischemia in cardiac tissue, reporter constructs containing the normal or mutant EPO 5'UTR fused to the Firefly luciferase cistron were expressed in H9c2 (heart myoblasts) and C2C12 (muscle myoblasts) cell lines.

Luminometry assays revealed that the EPO uORF represses translation of the main ORF in both cell lines. Under chemical ischemia, EPO uORF-mediated translation repression is specifically released in muscle cells. In response to chemical hypoxia, translational derepression occurs in both cell lines. We are currently exploring additional mechanisms through which EPO cardioprotection effects are regulated at the translational level.

Less is More: an overview on the use of RNAi as a tool to achieve Substrate Reduction in Mucopolysaccharidoses

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Mucopolysaccharidoses (MPSs) are a subgroup of Lysosomal Storage Diseases (LSDs) caused by dysfunction in enzymes responsible for the intralysosomal degradation of glycosaminoglycans (GAGs). Given their complex nature and the limitations of available therapies, the shift towards the development of combination treatments to counteract more effectively the pathological burden of these disorders is in the agenda of current research.

We consider that treatment strategies relying on RNA interference (RNAi), as well as in other RNA-based methodologies, may be feasible and particularly promising in the context of a synergistic combinatorial therapeutic approach. Therefore, we have designed an RNAi-dependent strategy based upon the selective downregulation of genes involved in the biosynthesis of GAGs, which is currently under evaluation. Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying MPSs' symptoms. Taking advantage of the RNAi technology potential, we have designed and assayed specific siRNAs targeting genes on those biosynthetic cascades to decrease the levels of production of each one of the four substrates: dermatan sulphate (DS), heparan sulphate (HS), keratan sulphate (KS), and chondroitin sulphate (CS). Their efficiency is currently being evaluated in vitro.

Here we present an overview of the preliminary results of this project and unveil its next steps towards a full characterization/evaluation of its potential therapeutic effect.

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P9

RAC1b impact on cancer-associated cellular processes – A comparison between normal and cancer thyroid tissues

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The overexpression of RAC1 splicing variant RAC1b was previously shown to be associated with poorer clinical outcomes in follicular cell-derived thyroid carcinomas. Nevertheless, the mechanisms influencing the pro-tumorigenic potential of RAC1b overexpression in thyroid remain unclear. In the colon, RAC1b was shown to impact on cancer-associated cellular processes via the activation of NF-kB and cyclin D1. Here, we aimed to ascertain the effect of RAC1b in the activity of NF-kB and cyclin D1 in different thyroid cell systems.

We selected one normal (Nthy) and three PTC-derived (K1, BCPAP, TPC1) cell lines and tested the activity of NF-kB and Cyclin D1 luciferase reporters in the presence and absence of RAC1b ectopic expression. RAC1b overexpression induced a significant increase in the activity of both reporters in both K1 and Nthy. However, in Nthy, RAC1 induced a more pronounced response than RAC1b, whereas in K1 it was RAC1b that induced a stronger response. Additionally, in BCPAP and TPC1 cells no significant reporter variations were observed upon RAC1b overexpression, possibly because the NF-kB pathway was found constitutively active in these cells.

These results indicate that RAC1b effect on NF-kB and Cyclin D1 may differ between normal and cancer thyroid tissues, as well as between different thyroid cancer cell systems, which point out the relevance of a specific signalling background.

P10

An integrative system biology approach for dissecting Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is characterized by a wide spectrum of behavioral presentation. Many genetic factors are implicated in ASD, however their role in the heterogeneous ASD phenotype remains elusive. Using data mining-based integrative approaches, we seek to identify patterns of association between ASD phenotypic subgroups and altered biological processes inferred from CNVs targeting brain genes. Analysis of ASD clinical data from 2067 patients, using Agglomerative Hierarchical Clustering, identified three distinct phenotypic clusters. These clusters differed in overall adaptive behaviour profiles (assessed by Vineland Adaptive Behavior Scales) and verbal status (assessed by Autism Diagnostic Interview-Revised). In Cluster 1, 72% of the individuals presented dysfunctional patterns of adaptive behavior, while all were verbal; Cluster 2 represented the subgroup with the most severe clinical presentation, with all patients non-verbal and 87% showing dysfunctional adaptive behaviour profiles; All patients in cluster 3 were verbal and only 56% of individuals exhibited dysfunctional adaptive behaviour, indicating a less severe phenotype.

In the same ASD subjects, analysis of rare CNVs targeting brain genes, using enrichment methods, predicted 21 statistically significant biological processes; several of these, like neuron-neuron synaptic transmission and nervous system development, are consistent with reported literature for ASD.

We are currently performing data mining using Classification And Regression Trees (CART) to uncover associations between the identified phenotypic clusters and brain biological processes. Our final objective with this approach is to develop a novel integrative method, allowing the prediction of clinical outcome from biological processes defined by genetic alterations, while further understanding ASD disease mechanism.

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P11

Stimulation of RAC1/PAK1 signalling upregulates DNA damage repair genes via STAT5 stimulation of BCL6 repressed loci

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Colorectal cancer is one of the most prevalent types of cancer worldwide. The GTPase RAC1 and its effector PAK1 have been found overexpressed or hyperactivated in colorectal cancers, particularly those with more aggressive and invasive features, leading to unfavourable clinical prognosis, often resulting from chemoresistance. Previously, we described a new signalling pathway in which activation of RAC1/PAK1 signalling promotes a transcriptional switch between the BCL6 repressor and the STAT5 transcriptional activator at a restricted subset of gene promoters. Here we used a novel combinatory ChIP-Seq approach for the genome-wide identification of the BCL6/STAT5-switch target genes. Ontological enrichment analysis among the identified target genes revealed an overrepresentation of genes involved in DNA damage repair. Using the comet assay as a read out for the extent of DNA damage, we show that the activation of RAC1/PAK1 signalling significantly accelerates DNA damage repair through the upregulation of pivotal genes. This work highlights an additional role for the RAC1/PAK1 signalling axis that may contribute to the chemoresistant phenotype of aggressive colorectal tumours.

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P12

The interplay between nonsense-mediated mRNA decay (NMD) and the unfolded protein response (UPR) in myocardial infarction

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Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that recognizes and degrades mRNAs carrying premature translation-termination codons (PTCs), protecting the cell from potentially harmful truncated proteins. Furthermore, recent studies have demonstrated that NMD is also a mechanism of gene expression regulation. This feature is reflected on its ability to regulate the cell response to many stress conditions, such as endoplasmic reticulum (ER) stress, hypoxia, reactive oxygen species, and nutrient deprivation. Stress conditions, specifically ER stress, has been related to myocardial infarction, a pathological state that occurs during ischemia, where nutrient and oxygen deprivation in the heart causes aggregation of proteins in the ER and the activation of the the three arms (ATF6, IRE1α and PERK) of the unfolded protein response (UPR) to mitigate the stress and avoid cell death. Given that NMD was seen to be able to regulate the UPR and to protect cells from death during ER stress, in this work we intend to study the impact of NMD in the PERK-mediated response to ER stress induced by ischemia during myocardial infarction, and its impact to the pathophysiology of this disease. For this purpose, differentiated H9c2 cells will be used as a model of cardiomyocytes, which will help us to dissect the crosstalk between NMD and UPR in myocardial infarction-mimicking conditions. By now, we have already established the differentiation protocol for the H9c2 cell line in order to obtain mature cardiac-like cells, and we are now optimizing and establishing the experimental conditions to further develop this project.

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P13

Characterization of Ezrin-mediated stabilization of rescued F508del-CFTR at the surface of airway cells

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Cystic Fibrosis is an autosomal genetic disease, caused by mutations in *CFTR* (cystic fibrosis transmembrane conductance regulator), which encodes a chloride channel present at the surface of epithelial cells, namely in the respiratory system. The most frequent mutation, F508del, compromises CFTR synthesis, transport and activity, abrrogating channel function and impairing ionic homeostasis in the lungs. Consequently, patients have airway obstruction due to increased mucus viscosity, which favours chronic infections and inflammation, ultimately leading to respiratory failure.

Compounds, such as VX-809 (lumacaftor), were described to partially correct the folding and trafficking defects of F508del-CFTR in vitro. However, these compounds produced only marginal effects in clinical trials. Our lab showed that these may relate to a decreased stability of the rescued channels at the cell's surface due to a failed interaction with the actin-binding adaptor protein Ezrin.

Here we further investigate why Ezrin fails to interact with rescued F508del-CFTR domain and show how the use recombinant peptides can counter this effect and enhance the functional recovery of rescued F508del-CFTR by chemical correctors.

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P14

Exploratory analysis of mutations targeting noncoding RNAs in autism

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Background and objective(s): Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication/interaction and by unusual repetitive and restricted behaviors. Heritability estimates indicate that genetic factors account for ~50% risk of ASD, sugesting a role of epigenetic factors, such as long noncoding RNA (lncRNA) and microRNA (miRNA), as modulators of genetic expression and clinical presentation. Our goal is to identify variants in lncRNA and miRNA *loci* that disrupt the function of target genes and modulating the high genotypic and phenotypic heterogeneity characteristic of ASD.

Materials and methods: We are screening for CNVs and SNVs encompassing lncRNA and miRNA *loci* in two large datasets: the Autism Genome Project (AGP), with CNV data from 2611 autism trios, and the ARRA Autism Sequencing Collaboration, with whole exome sequencing data (WES) from 3056 autism trios and 844 controls. Additionally we are using data from control population reported by two studies in the Database of Genomic Variant (DGV).

Results: An exploratory analysis indicates an excess frequency of CNVs targeting noncoding RNAs in ASD subjects from the AGP population (~12%) when compared to DGV control samples (~8%). Whole Exome Sequencing analysis further shows that ~15% of all the variants detected in exomes are targeting noncoding RNAs.

Discussion and conclusion(s): These findings highlight the importance of further exploring the role of these noncoding variants in ASD. We will now seek to understand the role of these regulatory factors in modulating the phenotypic heterogeneity of ASD in large patient datasets and multiply affected families.

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P15

New insights into how cancer cells regulate glucose uptake by protein phosphorylation

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Cancer cells require increased glucose supply to sustain proliferation. One mechanism involves increased expression of glucose transporter (GLUT) genes. But insulin has revealed that protein phosphorylation is another key mechanism in glucose uptake regulation: insulin binding to responsive cells triggers a signalling cascade with phosphorylation of TBC1D4, a negative regulator of endosomal GLUT trafficking, so that more transporters are inserted into the plasma membrane. Previous work from the host lab has identified the family of WNK protein kinases and shown that WNK1 can also phosphorylate TBC1D4 and promote GLUT translocation to the cell surface. Our objective is to understand the contribution of WNK1 to glucose uptake in colorectal cancer cells.

To characterize the role of WNK1, various colorectal cell lines were first cultivated with different glucose concentrations. Levels of GLUT1 at the cell surface were compared under these conditions and the effect of depleting WNK1 expression by siRNA determined. For selected conditions, key cell cycle or apoptotic marker proteins were analyzed by Western blot and revealed higher apoptotic and cell-cycle arrest phenotypes in WNK1-depleted cells cultured in low glucose medium.

In order to dissect key phosphorylation events involved in GLUT1 regulation, mass spectrometry analysis revealed that WNK1 specifically phosphorylates TBC1D4 at Ser704 and the functionally related TBC1D1 at Ser565. The respective phospho-mimetic mutants are currently being tested for their ability to increase GLUT1 translocation. Together, these studies will elucidate the molecular details regulating the translocation of glucose transporters in cancer cells and have the potential to identify novel therapeutic targets.

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P16

A Brand New World of Translation

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In recent years, non-canonical translation initiation mechanisms have been recognized as key factors in the development of different diseases such as cancer, as they present a survival answer during stress conditions by ensuring the expression of vital proteins.
Internal Ribosome Entry Sites (IRESes) were first discovered in viruses, and later in eukaryotes, as mRNA secondary structures capable of recruiting the ribosome to the vicinities of an initiation codon.
One of the most studied cancer-related genes, the p53 tumor suppressor gene, was found to possess on its mRNA an IRES capable of regulating the expression of the full length isoform, p53FL, and one of its isoforms, Δ40p53 differently by the interaction with MDM2 protein, an IRES trans-acting factor (ITAF) of p53.
Our aim is to study a shorter p53 protein isoform that lacks tumor suppressor behaviour acting instead as a cancer promoter (Candeias et al., 2016). One of our goals is to characterize the IRES associated with its expression. For that we will try to perform a sequencing reaction variant where the fragments to be sequenced result from uncomplete reverse transcription due to nucleotide-specific modification. Additionally, we will try to unveil new ITAFs by pulling-down the IRES and, consequently, associated factors, using two different methods: IRES biotinylation and MS2 tagging. Furthermore, we intend to find new IRESes by pulling-down MDM2 and possible bounded mRNAs followed by RNA-sequencing in order to identify them.

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P17

The applicability of the low-density lipoprotein cholesterol gene score in the Portuguese population

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Aim: Previous studies have demonstrated that the co-inheritance of LDL-C-raising alleles from 6 SNPs is associated with the Familial Hypercholesterolaemia (FH) phenotype. Here we investigate the applicability of the LDL-C genetic risk score in the Portuguese FH population.

Methods: Overall, 1320 DNA samples from a Portuguese population study (eCOR) and 262 from the Portuguese FH study (111 FH with known mutation (FH+), and 151 FH with no known mutation (FH-)) were genotyped for the 6 LDL-C genetic risk score SNPs. The LDL-C weighted score was calculated as previously described. The eCOR cohort was used to determine the score values for the healthy Portuguese population. Polygenic hypercholesterolaemia was defined as a score above the top quartile.

Results: FH- (0.68±0.21) and FH+ (0.72±0.19) patients had significantly higher score values than eCOR (0.62±0.22) (P<<0.001), a large proportion being in the top quartile (FH-: 38%, FH+: 31%). FH- patients had higher score values than FH+, although not significantly (P=0.09). Also, less FH- patients were in the bottom quartile than FH+ (11% *vs* 20%, P=0.035). Comparison of the UK control "Whitehall" sample and eCOR mean score values showed no significant differences (0.63±0.22 *versus* 0.62±0.22 [CI95%=0.61-0.63]).

Conclusion: The LDL-C genetic risk score was validated in the Portuguese population, and revealed that almost half of the FH- patients could have polygenic hypercholesterolaemia, while a small part might have unknown variants in a FH associated gene or in a new gene and should be investigated by exome sequencing. This study is ongoing.

P18

The DIS3 proteins family: role in the human transcriptome regulation and CRC tumorigenesis

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The final step of eukaryotic mRNA degradation proceeds in either a 5'-3' direction, catalyzed by XRN1, or in a 3'-5' direction catalyzed by DIS3, DIS3L1 (the catalytic subunits of the exosome) and/or DIS3L2 (exosome-independent). Important findings over the last years have shed a new light onto the mechanistic details of RNA degradation by these exoribonucleases. In addition, it has been shown that they are involved in growth, mitotic control and important human diseases, including cancer. With the aim of analyzing how DIS3, DIS3L1 and DIS3L2 regulate the human transcriptome, each one of these nucleases was depleted by RNA interference in HeLa cells and levels of several reporter mRNAs was monitored by RT-qPCR. Our results show that these exoribonucleases are target specific and not directly involved in a particular mRNA surveillance mechanism. In parallel, our bioinformatics analysis of available transcriptomic data from cells depleted of DIS3L1, DIS3L2, XRN1, or UPF1 (which has a central role in nonsense-mediated mRNA decay) has shown some, but not full, redundancy among the transcripts regulated by these nucleases, which supports our experimental data. Presently, we are exploring the molecular mechanisms underlying our observations and looking for relationships between their specific targets and their potential role in tumorigenesis.

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P19

Human AGO1 5'UTR mediates an eIF4G-enhanced but cap-independent mechanism of translation initiation

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Argonaute 1 (AGO1) is an essential effector in RNA-mediated gene silencing pathways. It regulates developmental control and stem cell maintenance, and is related to tumourigenesis. Such functions suggest its expression must be tightly regulated and, most likely, at protein synthesis level. Thus, we investigated whether AGO1 expression is controlled by alternative mechanisms of translation initiation. For that, we cloned the *AGO1* 5'UTR in a bicistronic luciferase vector upstream the downstream cistron (Firefly luciferase [FLuc]), and transfected HeLa cells with this construct. We observed a significant increase in FLuc expression levels compared to those from Renilla luciferase (upstream cistron) in cells transfected with the *AGO1* 5'UTR-containing constructs compared to those transfected with the empty transcript. Under cap-dependent translation initiation-impairing conditions, we saw that the identified cap-independent translation activity was enhanced upon knock-down of eukaryotic initiation factor (eIF) 4E, the cap-binding protein. However, inhibiting the eIF4G–eIF4E interaction significantly reduces such activity, suggesting *AGO1* 5'UTR-mediated translation may be dependent on eIF4G. Furthermore, in cells transfected with *in vitro* transcribed, capped and polyadenylated bicistronic *AGO1* 5'UTR-containing mRNA, the relative FLuc expression levels did not increase significantly, indicating that *AGO1* 5'UTR cannot mediate internal cap-independent translation initiation when it does not go through a nuclear experience. Nonetheless, in cells transfected with cap-lacking monocistronic transcripts, relative FLuc expression levels mediated by the *AGO1* 5'UTR were significantly increased. These results indicate that *AGO1* 5'UTR sequence mediates a non-canonical cap-independent eIF4G-dependent mechanism of translation initiation that seems to be enhanced by a free 5' end.

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P20

Expression of human UPF1 is regulated by a cap-independent translation initiation mechanism

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Gene expression comprises several intertwined steps. Translation initiation, which, under normal circumstances, is mostly cap-dependent, can also occur via a cap-independent mechanism, which drives protein synthesis under stress conditions impairing canonical translation initiation. Human up-frameshift 1 (UPF1) is a key-protein involved in nonsense-mediated mRNA decay, telomere replication and homeostasis, and cell cycle progression. These crucial UPF1 functions suggest its tight gene expression regulation. To test whether *UPF1* 5' untranslated region (5'UTR) mediates cap-independent translation, we cloned the *UPF1* 5'UTR in a bicistronic luciferase vector upstream the downstream cistron (Firefly luciferase [FLuc]), and transfected cervical and colorectal cancer cell lines with this construct. We observed a significant increase in FLuc expression levels compared to those from *Renilla* luciferase (upstream cistron) in cells transfected with the *UPF1* 5'UTR-containing constructs compared to those transfected with the empty transcript. To find which sequence segments are required for mediating cap-independent translation, we performed a deletional and mutational analysis of the sequence and verified that cap-independent translation was ceased when the first 100 nucleotides, or the last 125, were absent or altered. Also, such activity is maintained under canonical translation initiation-impairing conditions, such as hypoxia or endoplasmic reticulum stress. We also produced *in vitro* cap-lacking monocistronic *UPF1* 5'UTR-containing transcripts and observed a significant increase in relative FLuc expression levels in cells transfected with them.

These results indicate that *UPF1* 5'UTR mediates cap-independent translation initiation. Understanding this mechanism and its biological relevance might provide tools for developing new therapies for UPF1 deregulation-associated diseases, such as cancer.

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P21

Identification of novel biomarkers to distinguish polygenic and monogenic dyslipidemia

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Dyslipidaemia is one of the major cardiovascular risk factors. When it is characterized by a single gene mutation - monogenic dyslipidaemia – patients present the most severe phenotype and the earlier they are diagnosed, more successful the prognosis is. However, most hyperlipidemic patients appear to have a polygenic/environmental disease that can be controlled just by implementing a healthy lifestyle. Thus, distinguishing monogenic from polygenic dyslipidaemia is crucial for a prompt diagnosis, counselling and treatment. In spite of this, none of the current standard biomarkers (i.e. total cholesterol, LDL-c,HDL-c, triglycerides), can effectively make that discrimination between patients. This project aims to identify novel biomarkers that can establish these differences using an integrative approach.

Biochemical, clinical and genetic data of both dyslipidaemic and normolipidaemic subjects have been collected in the last 18 years. Using 618 subjects from Portuguese Familial Hypercholesterolemia (FH) Study as work data set, statistical tests allowed to identify biochemical parameters (i.e. TC, LDL, HDL, TG, apoA1, apoB, apoA1/apoB ratio, apoA2, apoC2, apoC3 and apoE) presenting significant differences between FH and non-FH subjects. Until the moment, ROC analysis has been performed for these parameters and those that better distinguish subjects are apoB/apoA1 ratio, apoB and LDL. Hence, new cut-offs for serum concentration of apoB/apoA1, apoB and LDL were determined. For adults these are 0,86 (apoB/apoA1); 141,54 (apoB); 209,80 (LDL). For under 18 subjects, new cut-offs are 0,66 (apoB/apoA1); 156,50 (LDL); 94,50 (apoB). Next step will envolve the application of machine learning methods as data analysis approach to provide a diagnosis algorithm.

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P22

Molecular characterization allowed the exclusion of inv(2)(p16.1;q14.3) as the cause of a severe congenital anomaly

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Background and objective(s): Congenital anomalies, a leading cause of infant mortality and heavy burden for patient and family, can be caused by chromosome rearrangements, which disrupt the genomic architecture at breakpoint regions. Presently, lack of an annotated non-coding genome hinders prediction of their phenotypic consequences. This study aims to clarify the causal relationship between a complex congenital anomaly observed in a subject with maternally inherited pericentric chromosome 2 inversion.

Materials and methods: The proband, harboring a cytogenetically identified inv(2)(p21;q21.1), presents severe psychomotor and developmental anomalies and autistic features. The parents are phenotypically normal. Genomic imbalances were analyzed by high-resolution oligonucleotide array. Large-insert whole-genome sequencing (liWGS) was used for breakpoints identification, followed by junction fragments amplification and familial segregation analysis by Sanger sequencing. Gene expression was profiled with high-resolution transcriptome array.

Results: A 590kb duplication at 2q21.1 was identified. The pericentric inversion was identified with high resolution by liWGS, redefining it as inv(2)(p16.1q14.3). Inversion breakpoints are flanked at 2p16.1 by *PNPT1* and *EFEMP1*, and at 2q14.3 by *TSN* and *CNTNAP5*. Expression levels of genes within duplication and inversion's breakpoint regions were not significantly altered.

Discussion and conclusion(s): The nonpathogenic 590kb duplication is of paternal origin. The inversion is identical in proband and his mother, confirming maternal origin. No phenotype-genotype correlation exist between these genomic alterations and proband's phenotype. Therefore, causal relationship between the congenital anomaly and this inversion is most likely excluded. Currently, no candidate gene was identified from the affected genomic regions. This study supports liWGS as an efficient method in the characterization of congenital disorders associated with chromosomal rearrangements.

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High risk Human Papillomavirus frequency and genotypes in an opportunistically screened Portuguese female population

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Background and Objective: Human Papillomavirus (HPV) is the etiological agent for cervical cancer and genital warts. Worldwide, cervical cancer is the second most common cancer in women and the high risk HPV (HR-HPV), namely HPV 16 and 18 are responsible for most of the cases. The objective of this study was to analyze the frequency of HR-HPV in a group of women referred for HR-HPV testing.

Methods: Clinical samples from 3117 women were perform by Cobas HPV test (Roche Molecular Systems, CA, USA), this real time PCR assay detected genotypes HPV 16 and HPV 18 and 'Other HR-HPV' (-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66 and 68). Positive samples for 'Other HR-HPV' were sequenced for HPV genotyping using MY09/11 primer´s.

Results: HR-HPV frequency was 20.8% (649/3117). Among the positive samples, 'Other HR-HPV' was the most common (72.8%; 473/649). HPV 16 and 18 were detected only in 14.9% (97/649) and 4.8% (31/649) of the cases, respectively. 7.4% (48/649) of the positive women were infected with more than one HPV (34 with 'Other HR-HPV' + HPV 16; 8 with 'Other HR-HPV' + HPV 18; 5 with 'Other HR-HPV' + HPV 16 + HPV 18 and 1 with HPV 16 + HPV 18). Sequencing of 'Other HR-HPV' is ongoing and the preliminary results shown the majority frequency for HPV 31 and 56 (13.8%) and 10.6% for the HPV 58.

Conclusions: In our study group the frequency of HR-HPV is high (20.8%), 27.1 % of these women were infected with HPV 16 or HPV 18 which is a high frequency. This study reveals the importance of the implementation of screening programs, and the advantage of using HPV detection.

P24

Molecular studies on HSV-1 and HSV-2 clinical isolates: replication rate, infection capacity and progeny

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Herpes simplex viruses (HSV) are ubiquitous host-adapted pathogens that cause a variety of different disorders. HSV-1 is traditionally associated with oro-facial infections and HSV-2 is mostly associated with genital ulcers; however this distinction is becoming less evident. Genital herpes is one of the most prevalent STI worldwide. A better understanding of the virus replication cycle is relevant to the pathogenesis of human diseases.

We aimed to evaluate the life cycle of various HSV genital clinical isolates with different viral loads in distinct host cell lines, giving special focus on capacity and efficiency of viral infection, regarding replication rate and progeny.

Confluent cell monolayers (Vero, Vero E6 and HeLa229) were infected with HSV clinical isolates at different MOIs, in 24-well plates and incubated for 30 hours at 37ºC and 5% CO_2. At different times-points of infection, the wells were scratched for kPCR and appropriate standard curves were generated by serial diluting plasmids cloned with both HSV single copy genes.

Results showed that both HSV isolates exhibited similar infection patterns regardless MOI, with DNA starting to be synthesized 6-12h post-infection; regardless HSV subtype, initial viral concentrations do not apparently affect adherence to any host cell line nor the generated progeny; Vero E6 cells seemed the most appropriate for HSV-2 infection; HeLa229 appeared to be the most suitable for HSV-1 infection for smaller inoculums; Vero had the worst viral growth results for both HSV subtypes; HSV-2 always displayed lower attachment capacities and growth rates, although higher progenies were seen in Vero E6 cells.

P25

Genetic Diversity of HIV-1 in individuals born in Guinea-Bissau living in Portugal

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Over time, the high level of mutation and genetic recombination rates of HIV-1 originated a large number of variants which were disseminated in human population. HIV-1 infection have a molecular distribution pattern in the world which is dynamic and influenced by the migration of the infected population. A high diversity of HIV-1 are described in Portugal which is increasing through time. Although still controversial, many studies suggest that recombinant forms of HIV may be more transmissible and virulent, accounting for the majority of the new infections worldwide. This highlights the importance of conducting molecular epidemiology studies and characterize the HIV infection. The study aims to analyse the genetic diversity of HIV-1 in a group of infected individuals born in Guinea-Bissau who are living in Portugal and compare the results with data previously obtained from infected natives resident in Guinea-Bissau. Genomic regions of HIV-1 (*env*, *nef,* PR and RT) were amplified by nested-PCR and sequenced by Sanger method from 37 extracted proviral DNA samples. The molecular analyses of sequences were performed using several bioinformatic tools which allowed to classify the virus by phylogenetic analysis. A prevalence of 81.1% was obtained for recombinant CRF02_AG, 5.4% for subtype A and 5.4% sub-subtype A1. In the remaining three cases (8.1%) was observed a different pattern of genomic recombination which could be described as unique recombinant forms (URF). The data obtained in the present study reflects the molecular epidemiology of HIV-1 describe in Guinea-Bissau which is similar to the obtained in a previous study conducted in natives from Guinea Bissau, however, the diversity is very low compared to the reported molecular epidemiology data in Portugal.

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P26

Inhibitory activity of *Solidago virgaurea* aqueous extract on Herpes simplex virus type 2

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Herpes simplex virus type 2 (HSV-2) is widely distributed and may be responsible for severe infections[1]. The most common antiherpetic is acyclovir and related drugs, however, long term treatments may result in viral resistance, leading to a continuous search for new/better therapeutic alternatives[2]. According to the WHO, plants are the best sources for obtaining a wide variety of drugs[3]. We aimed to evaluate the antiherpetic action of an aqueous extract from *Solidago virgaurea* L.(Asteraceae). We used Vero E6 cell cultures infected with HSV-2 and treated with the plant extract at different concentrations. Extract cytotoxicity was assessed by MTT-test; virucidal effect was evaluated by comparison of virus suspensions titers, incubated in contact/absence of the extract. Antiherpetic activity was investigated by treatment of infected cells during virus production, which revealed a mean yield reduction of 94% and an IC_{50} of 35.1 µg/mL. To evaluate the mechanisms that mediate the inhibitory effect of the extract, a kinetic of the first 7 hours of infection was performed with/without treatment. DNA samples from infected cells were subjected to PCR. Preliminary results showed the expected amplicon in treated/non-treated conditions. Amplification appears to start after 4 hours of infection but only increases under the non-treated conditions. This result is consistent with the low inhibition induced by the extract when it is added later than 4 hours post-infection. Our results suggest that *S. virgaurea* aqueous extract inhibits HSV-2 replication cycle, if added in the early phase of the infection, possibly by interfering with the viral DNA synthesis.

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P27

Implementation and validation of a low-volume protocol for large-scale small genome sequencing

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Background and objective(s): INSA is responsible for the sequencing task of the European project INNUENDO, which aims to deliver a standardized framework for the implementation of bacterial whole-genome sequencing (WGS) in public health surveillance, involving the sequencing of ~700 bacterial genomes from five foodborne pathogens (FBP). In order to reduce costs associated with sequencing, we aimed to implement a low-volume library preparation protocol without compromising sequence information and quality.
Materials and methods: Illumina Nextera XT libraries of three different strains from each of the FBP *Campylobacter jejuni* (~1.7 Mb, 30.4%GC) and *Escherichia coli* (~5.17 Mb, 50.6%GC) were prepared according to the standard protocol and a modified half-volume protocol. The libraries were paired-end sequenced using V3 (2 x 250 bp) chemistry on a MiSeq system (Illumina Inc.). Primary analysis was performed with Illumina Sequencing Analysis Viewer and FastQC. Then, the INNUCA pipeline was used for genome assembly, quality control and MLST prediction.
Results: The standard and modified protocols yielded equivalent results for all the tested strains regarding sequence quality (%≥Q30), GC content, median fragment length and percentage of alignment to the reference genome. The optimization had no impact on genome assembly and genetic relationships between isolates. Using this modified protocol, we successfully sequenced 331 strains from five FBP.
Discussion and conclusion(s): We validated a modified half-volume protocol for the reproducible preparation of Nextera XT libraries for small genome sequencing. This protocol allows a significant reduction in library preparation costs, making it more feasible for projects involving WGS of a large number of strains.

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P28

Imipenem resistance is associated with mutations in penicillin-binding proteins in a multiresistant clone of *Clostridium difficile*

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Background and objective(s): In the last decade, there has been an increase in the incidence and severity of *Clostridium difficile* infection (CDI) with a concomitant increase in the prevalence of hypervirulent and multiresistant strains. In this study, we characterized *C. difficile* strains isolated in Portuguese hospitals in order to identify genetic determinants of resistance to imipenem.
Materials and methods: Imipenem susceptibility testing by Etest was performed in 191 *C. difficile* strains isolated between 2012 and 2015. A group of imipenem-resistant and susceptible strains were selected for further phenotypic characterization and whole genome sequencing (WGS).
Results: Twenty-four (12.6%) strains were resistant to imipenem, 22 of which belonged to PCR ribotype (RT) 017 and had been isolated in a single hospital. These 22 strains showed a higher MIC compared with the two non-RT017 imipenem-resistant strains. Overall, RT017 imipenem-resistant strains presented reduced susceptibility to ertapenem and resistance to moxifloxacin, clindamycin, rifampicin and tetracycline. WGS identified 13 SNPs differentiating the 22 RT017 imipenem-resistant strains from three RT017 imipenem-susceptible strains isolated from a different hospital. Two of these mutations, identified in all 22 strains, affected the transpeptidase domain of two penicillin-binding proteins (PBP) (Ala555Thr and Tyr721Ser in CDM68_RS04280 and CDM68_RS05670, respectively) near the conserved motifs SxxK and SxN. One of the two non-RT017 imipenem-resistant strains presented the mutation Ala555Thr, and the other had a mutation also near to the functional motif SxN.
Discussion and conclusion(s): We describe for the first time the association of mutations in PBPs with the resistance to imipenem in *Clostridium difficile*.

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P29

Mechanisms of tigecycline-resistance among *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates

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Background and objective: The clinical efficacy of the most commonly used antibiotics against Gram-negative isolates is widely menaced, due to the increasing of multidrug-resistance phenotypes. The aim of this study was to characterize tigecycline-resistance mechanisms in order to understand how to extend its activity.

Materials and methods: Tigecycline-resistance due to efflux pump production was studied by molecular methods: *ramR* gene for 119 *Klebsiella pneumoniae* and *marR* gene for 10 *Escherichia coli* isolates. Fluoroquinolone and β-lactam resistance mechanisms of 623 *Enterobacteriaceae* isolates were reached by molecular methods targeting PMQR-, Class A-, B-, and D β-lactamases- and PMAβ-encoding genes. Efflux pump activity was also evaluated by using an Ala-Nap fluorescent assay.

Results: The molecular analyses of tigecycline resistance mechanisms revealed deletions, insertions and point mutations in the *ramR* gene that might contribute to the overexpression of AcrAB efflux pump in 63 out of 108 *K. pneumoniae* isolates showing reduced susceptibility to tigecycline. Considering the analyses of the *marR* gene from *E. coli* isolates (with or without tigecycline resistance), point mutations were detected. The results obtained with the Ala-Nap fluorescent assay were not discriminatory.

Discussion and conclusion: The results identified a great diversity of PMQR determinants and β-lactamases, such as ESBL and carbapenemases that are emerging in tigecycline resistant Gram-negative bacteria. The study also showed that tigecycline resistance through different mechanisms might contribute to multidrug-resistance scenarios, which are the cause of untreatable bacterial infections.

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P30

Carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Portugal: the new KPC-21 variant

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Background and objective: Here we report data from a six-month prevalence study on carbapenemase-producing *Enterobacteriaceae* (CPE) in the context of an European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE).

Materials and methods: This study included 94 *Klebsiella pneumoniae* and 10 *Escherichia coli* collected in 2013-2014. Antimicrobial susceptibility was performed according to EUCAST guidelines. Detection and characterization of oxyimino-β-lactam, and carbapenem resistance-encoding genes were performed by molecular approaches. KPC-21-producing *E. coli* was characterized by whole-genome sequencing. Genetic relatedness of isolates was investigated by PFGE and multilocus sequence typing (MLST). Subgroups of *E.coli* STs were analysed on the basis of sequence variation of the *E. coli* fimbrial adhesin gene *fimH*.

Results: During the study period, 67 isolates (61 *K. pneumoniae* and 6 *E. coli*) non-susceptible to carbapenems were identified in participant hospital laboratories. We identified 36 $bla_{\text{KPC-type}}$ (including one new variant: $bla_{\text{KPC-21}}$), 1 $bla_{\text{GES-5}}$, and 1 $bla_{\text{GES-6}}$ plus $bla_{\text{KPC-3}}$, alone or in combination with other *bla* genes. The remaining 28 isolates were non-susceptible to carbapenems due to association of PMAβ (CMY-2 and DHA-1) and/or ESBL (mainly CTX-M-15) beta-lactamases with porin deficiency. PFGE and MLST analysis showed an important diversity, with isolates belonging to distinct PFGE and STs profiles.

Discussion and conclusion: Portugal was one of the EuSCAPE participating countries that presented higher proportions of KPC-positive *K. pneumoniae*. However, although the percentage of CPE is still low in invasive infections, with unrelated hospital outbreaks detected, the number of inter-institutional transmission is increasing.

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P31

Characterization of *Haemophilus influenzae* invasive disease in Portugal: 2011-2016

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Background and objective(s): *Haemophilus influenzae* (Hi) although a commensal of the upper respiratory tract is also responsible for invasive disease. Six capsular types, a-f, have been identified to date, although most of the strains are non-capsulated (NC). We aim to characterize *H. influenzae* invasive isolates recovered in Portugal, between 2011 and 2016.

Materials and methods: During the study period, and as part of a laboratory-based surveillance system, 174 invasive isolates were received at our laboratory, from 36 Hospitals. Antimicrobial susceptibility was determined according to EUCAST guidelines. Capsular status and serotypes were characterized by PCR.
MLST was performed by amplifying and sequencing 7 housekeeping genes. Sequence type (ST) was assigned at https://pubmlst.org/hinfluenzae/. Presence/absence of selected virulence genes (*pilA*, *ompP5*, *hmw1A*/*hmw2A*, *HifA*, and *hia*) was determined by PCR, as previously described.

Results: Invasive disease was mainly due to NC strains (143/174; 82.2%); 31 isolates (17.8%) were capsulated and characterized as follows: 4 serotype a (2.3%), 21 b (12.1%) and 4 f (2.3%). Most strains were susceptible to antibiotics, with 12% (21/174) being β-lactamase producers. MLST (n=109) revealed high genetic variability among 80 NC isolates with 58 different STs (85%). Capsulated isolates were clonal: Hib-ST6, Hia-ST23 and Hif- ST124.

Discussion and conclusion(s): In Portugal, invasive disease is predominantly due to susceptible, highly genetically diverse NC strains. Preliminary results on the presence/absence of selected virulence genes show their important role in adhesion to host cells, especially in children. Ongoing surveillance of invasive Hi disease is needed to understand the burden of the disease and to develop public health prevention strategies.

P32

Genotypic characterization of *Staphylococcus aureus* isolates from human and animal origin

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Background and objective: Methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* isolates have been encountered in human and animal reservoirs. The objective of this study was to characterize *S.aureus* from humans, and to compare their genotypic characteristics with animal isolates.

Materials and methods: This study included a total of 136 *S. aureus* isolates: 119 recovered from patients admitted at Portuguese hospitals and 17 isolated from animals. Antibiotic susceptibility testing was performed to all *S. aureus* isolates according to EUCAST guidelines. Antibiotic-resistant genes were investigated. MLST/*spa*/*agr*-typing methods were applied to evaluate diversity and genetic relatedness.

Results: The majority of clinical *S. aureus* was MRSA, with reduced susceptibility to cefoxitin (*mecA* gene); no *mecC* was detected. Linezolid-resistant *S.aureus* harboured mutations in the domainV of the 23S rRNA and/or in *rlmN* gene, explaning the phenotype. We also identified that CC22-ST22-t032 and CC8-ST239-t037 lineages from hospital settings were linked to decreased susceptibility to daptomycin. One *S. aureus* isolate was hGISA (clone ST5/ST105-t002-*agr*2). Contrarily, all isolates from animals were MSSA. We identified CC398-ST398-t571 and CC130-t84 lineages previously described both in humans and animal infections. ST5 and ST34 were found in both reservoirs, while ST22/ST105 and ST121 were the most frequent STs identified among human and animal isolates, respectively.

Discussion and conclusion: The same ST and *spa* types in *S. aureus* from humans and animals suggest a potential dissemination between these two environments, which highlight the need to reduce the spread of this bacterium in different settings.

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P33

The hospitalization risk due to respiratory illness associated with a genetic variation at *IFITM3* in patients with influenza A(H1N1)pdm09 infection: a case-control study

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Background and objective: Recent studies suggest an association between the Interferon Inducible Transmembrane 3 protein (*IFITM3*) rs12252 variant and the course of influenza infection. However, it is not clear whether the reported association relates to influenza infection severity. The aim of this study was to estimate the hospitalization risk associated with this variant in Influenza Like Illness (ILI) patients during the H1N1 pandemic influenza.

Materials and methods: A case-control genetic association study was performed, using nasopharyngeal/oropharyngeal swabs collected during the H1N1 pandemic influenza. Laboratory diagnosis of influenza infection was performed by RT-PCR, the *IFITM3* rs12252 was genotyped by RFLP and tested for association with hospitalization. Conditional logistic regression was performed to calculate the confounder-adjusted odds ratio of hospitalization associated with *IFITM3* rs12252.

Results: We selected 312 ILI cases and 624 matched non-hospitalized controls. Within ILI Influenza A(H1N1)pdm09 positive patients, no statistical significant association was found between the variant and the hospitalization risk (Adjusted OR: 0.73 (95%CI: 0.33-1.50)). Regarding ILI Influenza A(H1N1)pdm09 negative patients, CT/CC genotype carriers had a higher risk of being hospitalized than patients with TT genotype (Adjusted OR: 2.54 (95%CI: 1.54-4.19)).

Discussion and conclusion: The *IFITM3* rs12252 variant was associated with respiratory infection hospitalization but not specifically in patients infected with Influenza A(H1N1)pdm09.

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P34

Hypertension: comparison of self-reported information and objective measures from the first Portuguese National Health Examination Survey (INSEF)

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Background and objective(s): This study aims to compare self-reported and examination-based hypertension prevalence in Portugal and to identify factors associated with measurement error in self-report.

Materials and methods: Portuguese National Health Examination Survey was conducted in 2015 on representative sample of 4911 adults aged 25-74 years. It combines blood pressure measurement, blood collection and interview. Self-reported prevalence of hypertension was defined as proportion of INSEF participants who reported medical diagnosis of high blood pressure. Examination-based prevalence of hypertension was defined as the proportion of those whose systolic blood pressure was at least 140 mmHg or diastolic blood pressure was at least 90 mmHg or who reported taking prescribed antihypertensive medication in two weeks prior the interview among all the survey participants. Logistic regression was used to estimate odds ratios (OR) of incorrect hypertension self-reports (any type of misclassification considering examination-based data as a gold standard) according to sex, age, education and general practitioner visit in the past year.

Results: The examination-based hypertension prevalence was 36.0% [95%CI:34.3-37.7] while self-reported prevalence was 25.7% [95%CI:23.9-27.5]. Incorrect report of hypertension was associated to male gender (OR=2.0, [95%CI: 1.5-2.8]), age between 45 and 54 years (OR=1.5, [95%CI:1.0-2.2], lack of general practitioner visit in the past year (OR=1.4 [95%CI:1.0-2.2]) and 1st cycle of basic education OR=2.0 [95%CI:1.3-3.0].

Discussion and conclusion(s): lf-reports underestimate prevalence of hypertension. Adding objective measurements to self-reported questionnaire improve data accuracy and allow better understanding of socioeconomic inequalities in health.

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P35

Move towards the understanding of the retirement effects on CCVDs

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Background and objective(s): Findings on the effects of retirement on health are conflicting, and few studies have focused on chronic diseases. The recent policies of increasing the retirement age in the OECD countries give the issue greater importance. The present study aimed to quantify the association between retirement transition (and retirement age) and the frequency of cerebro-cardiovascular diseases (CCVDs) in the Portuguese population.

Material and Methods: A cross-sectional study was performed, using data from the Survey of Aging and Retirement in Europe (SHARE) 2011 in Portugal. The odds ratios were estimated by Binary Logistic Regression with assessment for confounding and effect modification. Pensioners in retirement process (retired for 5 years or less) and not retired because of illness were considered.

Results: Retirement had negative effects on self reported heart disease, but only in non-hypertensive individuals. Early retirees, not suffering from a heart disease, had a decreased probability of having a stroke one year or more after retirement. On the contrary, in individuals with Heart disease, early retirement represented a protective factor for stroke.

Discussion and conclusions: Mechanisms through which retirement could influence CCVDs remain unexplained. Retirement effects seemed to vary across the categories of CCVDs main risk factors, being beneficial in their presence and harmful in their absence. Having not been found in any other study performed before, this can represent a new hypothesis to be confirmed in the future. This is relevant for Public Health, mainly allowing the detection of risk groups from which policies could be directed.

P36

Health Literacy – a tool for Health Promotion

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Health Promotion is the process that enables citizens to create conditions to increase their ability to control the determinants of health, allowing health-based decision making, with the aim of improving it. Health Literacy (HL) is a relevant tool in the field of Health Promotion as it relates to the ability to deal with health information, with respect to its access, understanding, interpretation, evaluation, application and use in various situations and throughout the life cycle.

In 2012, the European Health Literacy Project Consortium (HLS-EU Consortium) assessed the HL level in eight European Member States. In this context, data was collected on the HL levels of a sample of the Portuguese population (n = 1180) following the methodology of the HLS-EU Consortium.

The national sample results demonstrated a limited prevalence of HL (representing 55.9% of the respondents), thus manifesting the second lowest level of HL, only preceded by Bulgaria, compared to the eight countries included in the evaluation promoted by the European Consortium.

In practical terms, low HL may lead to a higher number of hospitalizations and a more frequent use of emergency services, as well as a lower prevalence of preventive attitudes - individual and collective - in the health field. This means a lower quality of life. For this reason it is essential and urgent to place the promotion of HL on the agenda of public policies.

P37

Genetic variants of CYP2C9 and IL-6 on female infertility

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Background and objective(s): Infertility affects 15–20% of couples worldwide. Within the past decades, there has been a steady rise in the treatment of female infertility with several drugs.The cytochrome P450 (CYP) genes are oxygenases involved in estrogen biosynthesis and metabolism, generation of DNA damaging procarcinogens, and response to anti-estrogen therapies used in female infertility treatments. Interleukin-6 (IL-6) is a pleiotropic proinflammatory cytokine, highly expressed in the female urogenital tract and reproductive organs. It has been implicated in estrogen metabolism imbalance.

Materials and methods: DNA was extracted from urine sediments. LightMix Kit for the detection of CYP 2C9 alleles *2, and *3 and LightMix Kit for the detection of IL 6 G-174C were used with LightCycler 2.0 Instrument.

Results: In the present study we investigated polymorphic variants in CYP2C9 and the -174 G/C (rs1800795) promoter polymorphism of the IL-6 gene on a cohort of 10 infertile women. We found that 20% of the infertile women are carriers of the CYP2C9*3 allele, which is characterized by deletion of the entire CYP2C9 gene, and 20% are heterozigotic for CYP2C9*3. Also we found that 80% have the IL6 -174C variant, that is known to be associated with lower IL6 secretion.

Discussion and conclusion(s): These polymorphisms may represent potential biomarkers for female infertility. On the other hand, they may have prognostic significance, namely regarding the metabolism of drugs used in infertility treatments, something which will need to be addressed in further studies.

P38

Air pollution and its adverse impacts on human health: FUTURAR Project Review

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Air pollution causes adverse impacts on human health, including premature mortality and morbidity. Despite the decrease and air quality improvement in Europe and Portugal over the last decade, ozone and particulate matter atmospheric concentrations are still exceeding the legal standards. In this context, the FUTURAR project (http://futurar.web.ua.pt/en/project) is focused on the assessement of environmental and health impacts associated with air pollutant emission reductions for 2030 imposed by the new National Emission Ceilings Directive (NECD). This Directive sets national reduction commitments for five pollutants, sulphur dioxide, nitrogen oxides, volatile organic compounds, ammonia and fine particulate matter (PM2.5), responsible for acidification, eutrophication and ground-level ozone pollution which leads to significant negative impacts on human health and the environment. Together with a cost-benefit analysis, this project addresses policy-oriented research gaps, namely country-specific exposure-response functions for most important morbidity endpoints. By this approach the reference exposure-response functions suitable for Portugal will be used to estimate health impacts for each emission reduction scenario and produce reference maps for each selected health indicator. Health indicators include premature mortality from exposure to ozone and particulate matter, as well as additional relevant morbidity health indicators to be defined. This research addresses public health policy strategies to be taken at national and regional level by competent authorities to control the emissions of air pollutants using integrated assessment modelling tools for policy support of these clean air strategies. This presentation focuses on the literature review, data collection and critical analysis of exposure-response functions for the pollutants addressed by the NECD.

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Hazard assessement of metallic nanomaterials in human respiratory cells

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As the applications of nanomaterials (NM) have been exponentially increasing, serious concerns about their potential impact on human health have been raised and challenging questions to regulators came up. This work investigated the toxicity of widely used metallic NM, namely cerium dioxide (CeO_2, NM-212), titanium dioxide (TiO_2, NM-100 and NM-101) and barium sulphate ($BaSO_4$, NM-220).

A standardized protocol for NM dispersion was followed and the quality of the dispersion in the culture medium was evaluated by dynamic light scattering. A human alveolar cell line (A549) was exposed to 1-100 $\mu g/cm^2$ of each NM for cytotoxicity (MTT and clonogenic assays) and genotoxicity (comet and micronucleus assays) assessment.

A decrease in cells' proliferative capacity was detected after exposure to the two highest concentrations of CeO_2 for 7 days ($p=0.01$ and $p=0.002$, respectively) while the remaining NM were not cytotoxic. Concerning genotoxicity, TiO_2 NM significantly increased the level of DNA breaks but those lesions seemed to be efficiently repaired because no chromosome instability was detected by the micronucleus assay. The CeO_2 NM induced a two-fold (non-significant) increase of in the level of oxidative DNA. $BaSO_4$ NM was neither cytotoxic nor genotoxic under the tested conditions.

Although the present results contribute to the risk assessment of these NM, the real effects from human exposure, e.g., in the workplace, are still unclear. Thus, the implementation of high throughput methodologies to allow cost-efficient strategies and experimental models that better mimic *in vivo* responses is an urgent need to allow nanosafety studies to keep pace with innovation.

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An exposome approach to frailty in older adults

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The prevention of frailty in old age is one of the key actions identified in Horizon 2020 Framework Program as part of the Societal Challenges in Europe. Frailty is a multidimensional syndrome characterized by increased vulnerability and functional decline that may be reversed, but if not addressed leads to long-term disability and hospitalisation. The aim of the present study is a) to identify new cellular and molecular biomarkers associated with frailty, b) to evaluate the potential influence of physical exercise on the biomarkers studied, and c) to investigate if exposures during life course may affect the way we aged.

Several endpoints at genomic, epigenomic, endocrine and immunological levels will be assessed to evaluate frailty status. The association of frailty with biomarkers will be performed through a cross-sectional design comparing groups of older adults (≥65 years of age) classified as frail and non-frail according to Fried's criteria and that have accepted or not to participate in a 9-month exercise program. A follow-up assessment of biomarkers and Fried classification will be carried out approximately 9-months after the cross-sectional assessment. In addition, questionnaires compiling information on socio-epidemiological and clinical features, and an exposure history questionnaire will be administered. Furthermore, the association between frailty status and some known bio-accumulating exposure biomarkers will be investigated.

This study has clear and important benefits for Public Health as the early identification of people at risk of frailty will allow implementing preventive actions and specializing geriatric care, improving the quality of life in old age and reducing healthcare costs.

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Evaluating the influence of light intensity in *mcy*A gene expression and microcystins production in toxic strains of *Planktothrix agardhii* and *Microcystis aeruginosa*

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Cyanobacteria are phytoplanktonic organisms widely occurring in freshwaters, being frequently associated with the production of toxins, especially microcystins (MCs). MCs are produced non-ribosomally by a multienzyme complex (*mcy* genes). It has been reported that environmental factors, such as light intensity, can influence toxin production. The aim of this study was to assess the influence of light intensity in the transcription of the *mcy*A gene and corresponding production of microcystins in toxic isolates of *Planktothrix agardhii*, where little is known, and compare them to *Microcystis aeruginosa*. For that purpose, cultures were exposed to three different light intensities for 18 days at 20 ± 1ºC. The growth was followed daily using absorbance readings. Samples were collected at each growth stage for cell counting, microcystins quantification and RNA extraction. The level of transcripts was quantified by RT-qPCR and the relative expression determined. Microcystin concentration per cell was similar between light intensities in *M. aeruginosa* and over time, while in *P. agardhii* it was higher in the stationary phase at 4 µmol photons m^{-2} s^{-1}. There were differences in the expression of *mcy*A between the two species. In *M. aeruginosa*, the highest levels of expression occurred at 4 µmol photons m^{-2} s^{-1} in the adaptation phase, whereas for *P. agardhii* it was at 4 µmol photons m^{-2} s^{-1} in the exponential growth phase. Comparing the temporal evolution of *mcy*A expression and the microcystins content per cell there seems to be an increase of *mcy*A expression levels which precedes the increase of the microcystins content per cell, in both species. This trend needs further confirmation, however it could help foresee the toxicity peaks in freshwater reservoirs, which would be preceded by a peak in *mcy*A transcripts.

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P42

How important is the study of assay interference prior to nanotoxicity assessment? Case study with TiO_2 nanoparticles immobilized in nanokaolin substrates.

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Previous studies showed that the unique physico-chemical features of nanomaterials are responsible for unexpected interactions with toxicity assay components, indicating that standardized toxicity tests used for hazard assessment of chemicals might not be fully suitable for assessing the safety of nanomaterials. Thus, for a suitable assessment of the toxicity of the nanoparticles, the possible nanoparticle-assay interactions should be identified.

This work evaluated the possible interferences between 3 nanomaterials (TiO_2 nanoparticles, nanokaolin clay and TiO_2 nanoparticles immobilized in nanokaolin substrates) and the cytotoxicity (MTT, neutral red uptake (NRU), alamar blue (AB) and LDH) and genotoxicity (alkaline comet assay) assays. This was essential to identify which of them were suitable for toxicity assessment of these nanomaterials, and to obviate possible interferences by introducing alterations to assay protocols. Thus, two main sets of experiments were conducted: light-absorption and catalytic interferences. For the LDH assay, an additional experiment was carried out to understand the possible nanomaterials interference on the enzymatic activity of LDH. For the alkaline comet assay it was performed a lysis test to estimate the nanomaterials capacity to damage DNA.

The obtained results suggest that nanokaolin and TiO_2 nanoparticles immobilized in nanokaolin substrates were able to adsorb NRU and LDH assay components, decreasing the signal in both assays with increased dosage. As these interferences could not be eliminated by protocol alterations, only MTT and AB assays seemed to be suitable for further cytotoxicity studies. Regarding the alkaline comet assay, after a slight alteration to the protocol, this assay was appropriate for the genotoxicity evaluation of all tested nanomaterials.

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Assessment of DNA Damage on a group of professional Dancers during a 10-month Dancing Season

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Despite the numerous health benefits of physical activity, some studies have shown that above certain intensity level and duration it may induce oxidative stress in several structures, including DNA. The aim of the present study was to evaluate the effect of intense physical activity at DNA level, both basal and oxidative damage, in a group of professional dancers before and after a 10-month dancing season. A group of individuals from general population was also assessed to compare the level of DNA damage with ballet dancers before season. Study population consisted of a total of 28 healthy subjects, among which 14 were professional dancers and 14 were controls. The classical comet assay version was employed to measure the basal DNA damage and the enzymatic version was used to assess the oxidative damage at the level of purines. Results showed that in dancers the levels of oxidative DNA damage were significantly increased after dancing season. Furthermore, the pre-season levels were lower than those obtained from the general population, suggesting an adaptation of antioxidant system of dancers. Results of the present biomonitoring study emphasise the need of more effective measures in order to protect ballet dancers from potentially occupational-health risks related to intense regular physical exercise. Good practice campaigns may be crucial to decrease risk.

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Evaluation of chemopreventive effects of an hexanic extract prepared from *Morinda citrifolia* fruits

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The identification of bioactive natural compounds that are able to reduce the deleterious health effects of toxicants is of utmost importance. One of these toxicants is patulin (PAT), a common mycotoxin affecting fruits' integrity and representing a serious health concern. *Morinda citrifolia* (noni) is a tropical plant that has been used in traditional medicine mainly for its therapeutic properties. This work intended to explore the chemopreventive properties of the noni fruit, using an hexanic extract prepared and characterized in Brazil.

The potential capacity of the noni extract to reduce the cytotoxic and genotoxic effects of three compounds with dissimilar modes of action – PAT, ethyl methanesulphonate (EMS) and hydrogen peroxide (H_2O_2) – was evaluated in a liver-derived human cell line (HepG2 cells) through the MTT and the Comet assays, respectively.

The results showed that cells' pre-exposure to the extract followed by co-exposure to PAT, EMS or H_2O_2 was able to significantly reduce the level of cytotoxicity and genotoxicity induced by the toxicants alone. When compared to caffeic acid, a recognized antioxidant, the noni extract revealed a capacity to reduce both patulin- and H_2O_2-induced cytotoxicity, suggesting that its chemopreventive properties do not rely exclusively on an antioxidant potential.

In conclusion, this study showed that the hexanic fraction of the *Morinda citrifolia* fruit displays a promising chemopreventive action against several toxic agents that deserves further investigation. The properties of other noni fruit fractions or extracts from other parts of the plant (e.g., leaves and roots) also need to be explored.

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P45

Pathogens in ornamental waters: A follow up study.

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Ornamental waters of easy access and populated with animals are quite attractive and can hide threats to human health. Here we evaluated the microbiota of ornamental waters in a Lisbon park. Water and biofilm samples where collected, in 2 lakes (L1-L2) and ornamental fountains (L3-L4) in February/2015. In May/2015 and monthly during a year (starting March/2016) samples from L4 where collected. Microbiota identification was performed as described previously[a]. Biofilm assembly was monitored by crystal violet assay and SEM[b] and antibiotic susceptibility was performed by conventional methods. The results of the first water sampling (Feb/2015) revealed the presence of *Enterobactereaceae* and non-fermentative oxidase-positive bacteria. Fountains and lakes presented different microbota being the highest diversity found in L1 hosting a duck population. This result suggested the existence of an interplay between animal inhabitants and microbiota which was confirmed by the second sampling of L4 (May/2015). Between the 2 sampling events a fish population was introduced and the microbiota was completely altered with the appearance of a typical fish pathogen (*Aeromonas* spp). This tendency was also confirmed over 2016. *K. pneumoniae* and *Aeromonas spp*., present as planktonic and biofilm organized bacteria in 2015 showed an enhanced ability to assemble biofilms *in vitro* at 25 °C than at 37 °C. Bacteria recovered from biofilm showed an increased antibiotic resistance compared to planktonic counterparts. The pilot study conducted during 2015 and the follow up study (still in progress) support a periodic control of ornamental water microbiota as simple preventive measure to avoid potential health issues.

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P46

Toxicity of rutile TiO_2 nanoparticles immobilized in nanokaolin nanocomposites on human hepatic HepG2 cell line.

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Immobilization of nanoparticles on inorganic supports has been recently developed, resulting in the creation of nanocomposites, which is expected to present fewer toxic effects than most nanoparticles in biological systems, due to their larger size. Concerning titanium dioxide nanoparticles (TiO_2 NPs), these have already been developed in conjugation with clays, but so far there are no available toxicological studies on these nanocomposites. This work evaluated the hepatic toxicity of nanocomposites (C-TiO_2), constituted by rutile TiO_2 NPs immobilized in nanokaolin (NK) clay, and its individual components.

These nanomaterials were analysed by means of FE-SEM and DLS analysis for physicochemical characterization. HepG2 cells were exposed to rutile TiO_2 NPs, NK clay and C-TiO_2 nanocomposite, in the presence and absence of serum for different exposure periods. The cytotoxicity was evaluated by using MTT and AB assays, while the genotoxicity was assessed by the alkaline comet assay.

A decreased cell viability was observed after exposure to all studied nanomaterials, while an increased DNA damage in HepG2 was observed in the absence of serum proteins after shorter exposure periods and in their presence for longer exposure period.

Although the immobilization of nanoparticles in micron-sized supports could, in theory, decrease the toxicity of single nanoparticles, the selection of a suitable support is essential. The present results suggest that NK clay is not the appropriate substrate to decrease TiO_2 NPs toxicity. Therefore, for future studies, it is critical to select a more appropriate substrate for the immobilization of TiO_2 NPs, in order to decrease their impact in the environmental and human health.

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Deciphering the toxicity of polycyclic aromatic hydrocarbons in HepG2 cell line.

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Polycyclic Aromatic Hydrocarbons (PAHs) are persistent pollutants present in the environment with known mutagenic and carcinogenic properties. In the present study the effect of exposure to single or multiple doses of benzo[a]anthracene (BaA), pyrene (Pyr) and three HPAHs (1-ClPyr; 1-BrPyr and 7-ClBaA) were evaluated in a liver-derived human cell line (HepG2). Cytotoxicity as accessed by the classic MTT and neutral red showed a mild toxic effect in response to single or multiple dose exposure for up to 72h; except for multiple dose exposure to BaA and 7-ClBaA (cumulative concentration of 4 μM) and single exposure to 10 μM BaA. Furthermore, a selective mitochondrial and lysosomal toxicity was observed for Pyr and BaA series, respectively. In order to understand the underlying molecular mechanisms responsible for this effect, ROS production, mitochondrial membrane depolarization, lysosomal pH, DNA fragmentation and apoptosis mediators were evaluated after exposure to single PAHs doses. All compounds were able to trigger oxidative stress after 24h as measured by catalase activity and a good correlation was found between mitochondrial membrane depolarization, lysosomal pH increase and MTT and neutral red assays, respectively. The evaluation of cell death mediators showed that caspase-3/7 but not anexin-V pathways were involved in toxicity triggered by the studied compounds. In conclusion, the studied PAHs, especially 1-BrPyr and BaA, exhibit cytotoxic effects when accumulated, and may have adverse effects to humans after long periods of exposure.

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House dust fungal communities' characterization: a double take on the six by sixty by six project (6x60x6)

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Background and objective(s): Fungi are a group microbes that are found with particular incidence in the indoor environment. Their direct toxicity or capability of generating toxic compounds has been associated with a large number of adverse health effects, such as infectious diseases, allergies and other toxic effects. House dust is a time integrative matrix easy to obtain and has been recommended for epidemiological studies on human exposure to environmental contaminants. This study aims to quantify and identify the fungal community in house dust samples collected using two different methodologies: active and passive sampling.

Materials and methods: Sampling was performed as part of the ongoing 6X60X6 Project in which six houses from Covilhã (Portugal), with building dates representative of six decades, were studied for a period of sixty days. House dust samples were collected by active – vacuum cleaner bags – and passive – dust settled in petri dishes – ways.

Results: When compared sampling methodologies, the active sampling showed higher amounts of Colony Forming Units (CFUs) per gram of dust. Regarding the taxon characterization, the passive sampling method proved to be more effective for the fungi's identification. The most frequent genera found overall were *Aspergillus sp.*, *Penicillium sp*., *Cladosporium sp*., *Alternaria sp*. and yeasts.

Discussion and conclusion(s): These results are consistent with results found in previous studies. House dust is a suitable matrix for fungal communities' characterization. Active sampling is able to integrate the dust borne fungi of the entire household and should be complemented with passive sampling for a better comprehension of the indoor fungal communities in future monitoring studies.

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Acrylamide mitigation in rye and oat bread

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Background and objective(s): Acrylamide is a carcinogenic substance for animals and to humans. The harmful effect of such compound was later confirmed, and recently the acrylamide was considered a neurotoxic and genotoxic substance[1]. This contaminant has been found in carbohydrate-rich foods since 2002[2]. There are many studies regarding several mitigation strategies, however it is need to change the manufacturing processes[3]. The aim of the study was to reduce the acrylamide content in bread.

Materials and methods: It was selected oat and rye breads which are consumed ones daily by the population. The confection trials of rye and oat breads were made with the addition of 15 polyphenols-rich additives separately. For detection and quantification of acrylamide was used the ultra-efficiency liquid chromatography coupled to a mass detector.

Results: In oat bread was found that only one additive allowed 77.8% reduction of acrylamide. Such value was below of the indicative value published by EFSA, 150 µg/kg[2]. In rye bread was obtained a maximum reduction of 79%. Comparing the rye bread results with the indicative value of EFSA, only one bread was lower than 150 µg/kg[2]. However, further studies on the quantity of additives added are needed, due to the close results of the indicative value.

Discussion and conclusion(s): In general, there was a wide variability in acrylamide levels among the two breads. These differences can be caused by the nutritional composition of each flour. A more detailed study of the mechanism of the reduction of these additives in different nutritional composition flours is, therefore, necessary.

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Iodine content in food: comparison between Azores and continental Portugal

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Background and objective(s): Iodine is an essential trace element for the synthesis of thyroid hormones, in particularly for pregnant women and children. Clinical studies in Portugal have demonstrated the existence of a generalized deficiency in iodine of pregnant women and with school-age children particular severity in the archipelago of Azores [1, 2]. The main cause of iodine deficiency is low levels of iodine in foods [3]. A general lack of awareness within the Portuguese population about the importance of iodine in the diet has also been identified [4, 5]. Thus, in this work we address the characterization of iodine in foods available in Azores and continental Portugal to promote the consumption of iodine rich foods.

Materials and methods: A total of 110 samples 60 from Azores (Az) and 50 from Portugal (cPT) were analyzed as consumed. The iodine content in dairy products, fruits and vegetables was determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Results: Analysed foods showed large variation in iodine content, with values of 255µg/l (cPT) and 133µg/l (Az) for milk, 275µg/kg (cPT) and 117µg/kg (Az) for cheese. However vegetables collected in Azores presented higher iodine content than those purchased in continental Portuguese supermarkets.

Discussion and conclusion(s): All results are sound supported in metrological tools and aligned with other studies published in the literature. This is a premise to estimate the iodine intake of Azores and Continental population and a contribution to understand the role of this intake in the risk of Iodine Deficiency Disorders observed mainly in Azores region.

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Nutritional and Inorganic contaminants profiles of Shiitake mushrooms (*Lentinula edodes*) growing under different conditions

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Background and objective(s): Different factors affect nutritional composition and contaminant profile of shiitake mushroom such as: cultivation process, strain used, method and fruiting conditions, species of wood used for the underlying base structure, and geographic origin. [1] [2] [3] [4] This study evaluated nutritional composition and heavy metals profile of Shiitake mushrooms (*Lentinula edodes*) obtained by different cultivation processes and areas.

Materials and methods: Three samples of organically cultivated *Shiitake donko* mushrooms were collected (A1, A2, A3).A1 e A2 were produced in Amarante by a similar process, using the sprinkler method to induce the fruiting coming from spawns produced by a Portuguese and a Belgian company, respectively. A3 from a production located in Sintra. Fruiting was induced by dipping. Macronutrients, Vitamins, Mineral and Trace elements were determined by standardized methods. Heavy metals by ICP-MS and Arsenic Species by HPLC-ICP-MS. The statistical design was completely randomized with three replicates per treatment and analysis of variance with an F-test using the Statistical Analysis Software System (SAS).

Results: Macronutrient and mineral profile changes according to cultivation process and geographic area. Carbohydrate content ranged from 1.0 g/100 g (A1) to 6.7 g/100 g (A3). Total sugars ranged between 1.0/100g (A1) to 4.1 g 100 (A3)/g. A significant content of vitamin B2 in all samples was observed. A3 revealed superior mineral content. A2 presented highest arsenic content, without risk to consumer.

Discussion and conclusion(s): In conclusion, results obtained indicate shiitake mushroom as a dietary source of several nutrients, and the sample fructified by dipping (A3) seems nutritionally richer and chemically safer.

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Characterization of arsenic and selenium species in Portuguese diet as a contribution to selenium protective mechanism against arsenic toxicity.

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Background and objective(s): Selenium is an essential trace element for human health. Through selenoproteins, it participates in various biological processes such as antioxidant defence, thyroid hormone production and immune responses. The role of Se in the prevention of As – induced toxicity has been documented in several studies [1]. Se-dependent sequestration of As is suggested as a primary mechanism of interaction between Se and As toxic species. To understand the mechanisms involved in the Se/As interactions it is necessary to characterize their presence in foods as principal source of exposure[2]. The aim of this work was to evaluate Se Methionine (SeMet), Selenite (Se (IV)) and Selenate (Se (VI)) and Arsenite (As(III)), Arsenate (As(V)), Dimethylarsinic acid (DMA) and Arsenobetaine (AsB) in fresh and cooked foods representative of the Portuguese Diet.

Materials and methods: Speciation analysis was carried out by High Performance Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Before analysis, samples were extracted using specific extraction procedures for As and Se.

Results: More than 90% of the As present in fish samples was in the form of AsB while in rice samples results showed that 80% of the As was present as inorganic forms and 20% as DMA. SeMet was the major Se species in milk and fish samples accounting for 85-90 % of the total Se content. In cabbage Se (IV), Se (VI) and (SeMet) were found.

Discussion and conclusion(s): The results showed the suitability of the analytical procedures to achieve an accurate determination of As and Se species in these complex matrices.

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