3º Dia do Jovem Investigador 2022
Instituto Nacional de Saúde Doutor Ricardo Jorge

Organização

Conselho Diretivo do INSA

Conselho Científico do INSA

Comissão organizadora:

Presidente do Conselho Científico (Peter Jordan)

INSA ConVida (Pedro Henrique Fonseca, Vânia Gonçalves, Ausenda Machado, Diogo Ribeiro, João Pedro Xavier Santos, Juliana Inês Santos, Susana Jesus, Célia Ventura, Alexandra Nunes, Carina Menezes)

Comissão científica:

Helena Soares Costa, Elizabeth Pádua, Mafalda Bourbon, Luísa Romão

3º Dia do Jovem Investigador INSA 2022

8 de novembro de 2022

Programa

10h00 SESSÃO DE ABERTURA

Fernando de Almeida | *Presidente do Conselho Diretivo do INSA*

Peter Jordan | *Presidente do Conselho Científico do INSA*

10h15 CONFERÊNCIA INAUGURAL

Ser cientista: história e perspetivas para a Ciência em Portugal

Professor Carlos Fiolhais | *Universidade de Coimbra*

11h00 INTERVALO PARA CAFÉ

11h30 INVESTIGAÇÃO EM SAÚDE E NOVAS TECNOLOGIAS

Moderador: Peter Jordan | *Presidente do Conselho Científico do INSA*

Shotgun Proteomics evaluation of Obstructive Sleep Apnea (OSA) severity and response to Positive Airway Pressure (Cristina Valentim-Coelho | *Departamento de Genética Humana - INSA*)

Development of an extended next-generation sequencing panel for Familial Hypercholesterolemia phenotype (Ana Medeiros | *Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis - INSA*)

Performance of Sanger and NGS for the detection of resistance associated substitutions to HCV NS5A inhibitors in infected drug users (Diogo Ramos | *Departamento de Doenças Infecciosas -INSA*)

12h15 INVESTIGAÇÃO EM DOENÇAS INFECIOSAS

Moderador: Ausenda Machado | *Departamento de Epidemiologia – INSA*

Modelling the impact of vaccination strategies to control the COVID-19 epidemic in Portugal (Constantino Caetano | *Departamento de Epidemiologia - INSA*)

ReporTree: a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data (Verónica Mixão | *Departamento de Doenças Infecciosas - INSA*)

Multi-country 2022 outbreak of monkeypox virus: from the first viral genome sequence to phylogenomic characterization and microevolution (Joana Isidro | *Departamento de Doenças Infecciosas - INSA*)

13h00 ALMOÇO LIVRE

14h00 **Visita aos posters**

15h00 **INVESTIGAÇÃO EM DOENÇAS DEGENERATIVAS**

Moderador: Vânia Gonçalves | *Departamento de Genética Humana – INSA*

RNA as a promising molecule to treat a rare neurodegenerative lysosomal storage disorder (Juliana Santos | *Departamento de Genética Humana - INSA*)

Up-frameshift 1 (UPF1) internal ribosome entry-site-mediated translation and its importance in colorectal cancer (Adriana Elias | *Departamento de Genética Humana - INSA*)

Toxicity assessment of consumer-relevant nanomaterials in human barrier cells (Joana Pires | *Departamento de Saúde Ambiental - INSA)*

Pro-inflammatory cytokines induce changes in expression of tumour-related splice variant RAC1B in polarized colorectal cells (Joana Pereira | *Departamento de Genética Humana - INSA*)

16h00 INTERVALO PARA CAFÉ

16h30 **CONFERENCISTA CONVIDADA**

Moderador: Pedro Fonseca | *Departamento de Genética Humana – INSA*

Desenvolvimento de Carreira e Oportunidades

Mariana Santa-Marta | *Instituto Superior Técnico – Universidade de Lisboa*

17h00 **Entrega dos prémios (melhor comunicação oral e melhor poster) e Encerramento**

Conferência inaugural

Ser cientista: história e perspetivas para a Ciência em Portugal

Professor Carlos Fiolhais

Universidade de Coimbra

tcarlos@teor.fis.uc.pt

Carlos Manuel Batista Fiolhais nasceu em Lisboa em 1956 e doutorou-se em Física Teórica na Universidade Goethe em Frankfurt-Am-Main, na Alemanha, em 1982. Foi Professor de Física na Universidade de Coimbra, tendo terminado a carreira docente em julho de 2021. É autor de mais de 120 artigos científicos, um dos quais com mais de 7800 citações, e de mais de 450 artigos pedagógicos e de divulgação científica. É também autor de manuais de ensino de ciência, para todos os níveis de ensino, e de livros de divulgação de ciência, alguns traduzidos no estrangeiro, num total de 42 livros publicados.
Fundou o Centro de Física Computacional, o Centro Ciência Viva Rómulo de Carvalho e foi Diretor da Biblioteca Geral da Universidade de Coimbra. Colaborou com várias instituições dos media e museus de ciência e recebeu vários prémios e distinções, das quais se destaca a Ordem do Infante D. Henrique.

Conferencista convidada

Desenvolvimento de Carreira e Oportunidades

Mariana Santa-Marta

Instituto Superior Técnico – Universidade de Lisboa

mariana.santa-marta@tecnico.ulisboa.pt

Mariana Canelhas Palminha Santa-Marta licenciou-se em Biologia na Universidade de Lisboa e doutorou-se em Microbiologia na Faculdade de Farmácia em 2006. Desenvolveu trabalho de investigação na área da Virologia, em particular no estudo do HIV-1 até 2014. Desde 2015 até 2021 exerceu atividade como *Pre-Award Grants Manager*, tendo passado pela Fundação Champalimaud. Desde final de 2021 é responsável de atividades *Pre-Award* no Centro de Recursos Naturais e Ambiente (CERENA) e Centro de Estudos de Gestão do Instituto Superior Técnico (CEGIST). É uma experiente gestora de bolsas e financiamentos, habilitada em processos de candidatura, desde a identificação personalizada, divulgação de oportunidades de financiamento e desenvolvimento de propostas, até à negociação de candidaturas financiadas por agências de financiamento nacionais e internacionais.

Comunicações orais

O1

Shotgun Proteomics evaluation of Obstructive Sleep Apnea (OSA) severity and response to Positive Airway Pressure (PAP) treatment

Cristina Valentim-Coelho[1,2*], Hugo Osório[3,4], Fátima Vaz[1,2], Sofia Neves[1,2], Paula Pinto[5,6], Cristina Bárbara[5,6], Deborah Penque[1,2]

[1]Laboratório de Proteómica, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal; [2]ToxOmics – Centre of Toxicogenomics and Human Health, Universidade Nova de Lisboa, Portugal; [3]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; [4]Ipatimup – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal; [5]Serviço de Pneumologia, Centro Hospitalar Lisboa Norte, Portugal; [6]ISAMB-Instituto de Saúde Ambiental, Faculdade de Medicina, Universidade de Lisboa, Portugal.

OSA syndrome is characterized by recurrent episodes of apneas/hypopneas during sleep, leading to recurrent intermittent hypoxia and sleep fragmentation. Non treated OSA can result in cardiometabolic diseases. By 2DE-gel, we have demonstrated redox/oligomeric changes in OSA red blood cell (RBC) proteins that can be reverted/modulated by PAP treatment. Herein, we applied a shotgun proteomics to gain deeper insights into RBC homeostasis in OSA severity and treatment response.

RBCs from snorer patients as controls (n=23) and patients with mild (n=16) or severe OSA (n=17) before/after six months of PAP were selected from our biobank. Samples were lysed, haemoglobin depleted and alkylated/reduced before trypsin/ chymotrypsin digestion. Peptides were analysed by nanoESI-Orbitrap mass spectrometer and data by MaxQuant/Perseus bioinformatics.

From 1466 proteins identified, 397 with score ≥40 were selected for analysis. 47 and 82 proteins showed differential abundance ($p<0.05$) in mild and severe OSA RBC, respectively, compared with snorer RBC. In severe OSA, these proteins are mainly associated with glycolytic process, cadherin and NAD binding (ALDOC, ENO1, GAPDH, LDHA, LDHB, PGK1, PKLR, PRDX6). Oxygen transport, regulation of cell death and haptoglobin binding (HBA1, HBA2, HBB, HBG1, HBQ1) associated proteins were mostly changed in mild OSA. After PAP, changes in the abundance of some of these proteins was restored. In OSA, mostly in severe OSA, the PRDX2 was identified highly hyperoxidized in its catalytic Cys51 that decreased after treatment with PAP.

Further analyses will provide new insights into the RBC molecular mechanisms and PTMs associated with OSA severity and response to therapy.

Acknowledgements

Patients that voluntarily collaborated in this study. Project partially supported by the Harvard Medical School-Portugal Program (HMSP-ICJ/0022/2011), the ToxOmics (FCT-UID/BIM/00009/2013) and the Portuguese Mass Spectrometry Network (RNEM). CVC is recipient of the FCT doctoral scholarship FCT-SFRH/BD/133511/2017.

O2

Development of an extended next-generation sequencing panel for Familial Hypercholesterolemia phenotype

Ana Margarida Medeiros[1,2], Ana Catarina Alves[1,2], Mafalda Bourbon[1,2]

[1]Unidade de I&D, Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Background and objective(s)

Familial Hypercholesterolemia (FH) is a common genetic disorder of lipid metabolism with severely elevated LDL-C levels. Three primary genes are associated with FH (LDLR, APOB, PCSK9). Variants in FH phenocopies genes (*LDLRAP1*, *APOE*, *LIPA*, *ABCG5*, *ABCG8*) and LDL-C polygenic risk score (PRS) can mimic the FH phenotype. We present a new diagnosis method for FH and the overall genetic results of the Portuguese FH Study cohort.

Materials and methods

Individuals with clinical diagnosis of FH (Simon Broome criteria) were referred to our study (N=1005). Genetic diagnosis of FH included the 3 primary genes by Sanger sequencing (1999-2016). Since 2017, it is performed by an NGS panel that captures 8 genes and 6-SNPs of the PRS. Selected 274 cases, where no pathogenic variant was found in the 3 genes (1999-2016), were investigated using this panel.

Results

FH was genetically confirmed in 415 cases (406 heterozygous, 3 true-homozygous, 6 compound-heterozygous) carrying pathogenic/likely pathogenic variants in *LDLR* (94%), *APOB* (5%) and *PCSK9* (1%). Cascade screening in family members identified 581 heterozygous, 1 compound-heterozygous and 1 *LDLR-APOB* double-heterozygous. In the FH-negative cohort, individuals present heterozygous pathogenic variants in *ABCG5* (N=4) and *ABCG8* (N=1), heterozygous variants of unknown significance in *ABCG5* (N=19), *ABCG8* (N=10), *APOE* (N=3), *LIPA* (N=1) and *ABCG5-ABCG8* double heterozygosity (N=2). Additionally, 190 of these present high PRS.

Discussion and conclusion(s)

Overall, FH was confirmed genetically in 41% of the cohort. Heterozygous *ABCG5/8* variants and PRS may explain the phenotype of hypercholesterolemia in FH-negative patients. This extended NGS panel is important to identify FH/FH-phenocopies and therefore personalize each patient's treatment.

Acknowledgements

All participants and collaborators of the Portuguese FH Study (EPHF); AMM was supported by a PhD student grant (Ref. FCT: SFRH/BD/113017/2015) between 01-2016 and 02-2019.

O3

Performance of Sanger and NGS for the detection of resistance associated substitutions to HCV NS5A inhibitors in infected drug users

Diogo Ramos and Elizabeth Pádua

Reference Laboratory of HIV and Hepatitis B and C, Department of Infectious Diseases, National Institute of Health, Lisbon

Hepatitis C virus (HCV) may lead to severe liver damages, including cirrhosis and hepatocellular carcinoma. Due to their transmission routes (mainly by blood), the infection is prevalent in high-risk behavior groups such as injection drug users (IDU). The intra-host variability of HCV characterizes the infection, and the selecting driving forces in infected hosts, such as therapeutic pressure, could select variants with resistance associated substitutions (RAS) and, therefore, reduce treatment efficacy.
The aims of this study were to perform a molecular analysis of one therapeutic target (NS5A viral protein) in IDU with chronic Hepatitis C and naïve to antivirals treatment a) to confirm HCV genotypes/ subtypes in samples and b) to assess the presence of RAS in the amino acid sequences.
RNA extraction, PCR amplification, and two automatic sequencing methods [Sanger and Next-generation sequencing (NGS)] were used.
Phylogenetic classification of the sequences derived from both sequencing methods were concordant: 1a, 52.4%; 1b, 10.7%; 3a, 20.2%; 4a, 8.3%; 4d, 7.1%, and one genetic recombinant form (2k/1b) was also identified. RAS were found in 34.5% (29/84) of the analyzed samples using Sanger sequencing and in 42.9% (36/84) using NGS. A mixed infection by subtypes 1a and 3a was detected only by NGS.
The obtained data showed that NGS allows a more sensitive analysis of RAS in infected patients, which is crucial to achieve treatment efficacy. This strategy might contribute to the achievement of United Nations Goal 3 from the 2030 Agenda, regarding the elimination of Hepatitis C.

Acknowledgements
Rodrigo Coutinho, on behalf of Ares do Pinhal Association for Social Inclusion.
Luís Vieira, on behalf of the Technology and Innovation Unit of the National Institute of Health and **Miguel Pinto** from the Genomics and Bioinformatics Unit of the Department of Infectious Diseases of the National Institute of Health.

O4

Modelling the impact of vaccination strategies to control the COVID-19 epidemic in Portugal

Constantino Caetano[1,2], Luísa Morgado[2,3], Paula Patrício[4], João Pereira[1,3], André Torres[5], Andreia Leite[5,6], Ausenda Machado[1,5], Sónia Namorado[1,5,6], Ana Sottomayor[7], André Peralta[7], Baltazar Nunes[1,5,6]

[1]Departamento de Epidemiologia, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal; [2]Center for Computational and Stochastic Mathematics, Instituto Superior Técnico, University of Lisbon; [3]Department of Mathematics, University of Trás-os-Montes e Alto Douro, UTAD; [4]Centro de Matemática e Aplicações (CMA), FCT, UNL and Departamento de Matemática, FCT, UNL, Campus de Caparica, 2829-516, Caparica, Portugal; [5]NOVA National School of Public Health, Public Health Research Center, Universidade NOVA de Lisboa; [6]Comprehensive Health Research Center, Universidade NOVA de Lisboa; [7]Direção de Serviços de Informação e Análise, Direção Geral da Saúde, Lisboa, Portugal

Modelling studies emerged as a fundamental tool to inform pharmacological and non-pharmacological interventions during the COVID-19 pandemic. In this study we developed an age-structured SEIR deterministic model to measure the impact of vaccination strategies on the disease spread of COVID-19 in Portugal and also to study the possible effects of infection and vaccine-induced immunity waning. Data on the number of hospitalisations was used to calibrate the model. Other model parameters were obtained from COVID-19 literature and estimated from Portuguese case data. The vaccination simulations considered took into account seroprevalence data, current estimates on vaccine effectiveness and coverage by age-group. Vaccination scenarios presented possible trajectories of the future number of hospitalisations, given certain assumptions.
An analysis of the adequate effective reproduction number showed that non-pharmaceutical interventions should not be fully phased-out and instead be combined with vaccination strategies to keep transmission low. However, given the presence of immunity waning, booster shot strategies should be adopted in order to prevent disease burden on health services. The results obtained helped to inform public health policies to mitigate the burden of COVID-19 in Portugal.

Acknowledgements
The authors acknowledge financial support from the Fundação para a Ciência e Tecnologia - FCT through project 692 2ª edição Research 4 covid, project name "Projection of the Impact of Non-pharmacological real-time Control and mitigation measures for the COVID-19 epidemic" (COVID-19 in-CTRL) - project nº 692 from the 2nd edition of RESEARCH 4 COVID-19. The first author also acknowledges FCT within the PhD grants "DOCTORATES 4 COVID", grant number 2020.10172.BD. The second author also acknowledges FCT within projects UIDB/04621/2020 and UIDP/04621/2020. The third author also acknowledges FCT within the Strategic Project UIDB/00297 /2020 (Centro de Matemática e Aplicações, Universidade Nova de Lisboa).

O5

ReporTree: a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data

Verónica Mixão[1], Miguel Pinto[1], Daniel Sobral[1], Adriano Di Pasquale[2], João Paulo Gomes[1] and Vítor Borges[1]

[1]Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [2]National Reference Centre (NRC) for Whole Genome Sequencing of microbial pathogens: data-base and bioinformatics analysis (GENPAT), Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" (IZSAM), Teramo, Italy

Genomics-informed pathogen surveillance strengthens public health decisionmaking, thus playing an important role in infectious diseases' prevention and control. A pivotal outcome of genomics surveillance is the identification of genetic clusters and their characterization in terms of geotemporal spread or linkage to clinical data. This task often consists of the visual exploration of (large) phylogenetic trees and associated metadata, being time-consuming and difficult to reproduce. As such, we aimed to create an automated bioinformatics tool that facilitates the detection of genetic clusters and their linkage to epidemiological data.
We developed ReporTree, a flexible bioinformatics pipeline (available at https://github.com/insapathogenomics/ReporTree) designed for surveillance of multiple pathogens. ReporTree allows diving into the complexity of pathogen diversity to rapidly identify genetic clusters at any (or all) distance threshold(s) of a tree, identify regions of cluster stability (key step in nomenclature design), and further characterize them according to any relevant feature, such as timespan, geography or clinical status. Noteworthy, ReporTree currently plays a key-role in the routine surveillance and outbreak investigation of bacterial (e.g., *Listeria monocytogenes*) and viral (e.g., SARS-CoV-2 and Monkeypox virus) pathogens in Portugal, facilitating and accelerating the production of surveillance-oriented reports. For instance, besides its weekly usage for assessing the frequencies of SARS-CoV-2 variants (https://insaflu.insa.pt/covid19/), ReporTree is routinely applied to identify potential listeriosis outbreaks.
In summary, ReporTree is a pan-pathogen tool for automated and reproducible identification and characterization of genetic clusters that contributes to a sustainable and efficient public health genomics-informed pathogen surveillance.

Acknowledgements
This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

O6

Multi-country 2022 outbreak of monkeypox virus: from the first viral genome sequence to phylogenomic characterization and microevolution

Joana Isidro[1,*], Vítor Borges[1,*], Miguel Pinto[1,*], Daniel Sobral[1], João Dourado Santos[1], Alexandra Nunes[1,5], Verónica Mixão[1], Rita Ferreira[1], Luís Coelho[1], Daniela Santos[2], Silvia Duarte[2], Luís Vieira[2], Maria José Borrego[3], Sofia Núncio[4], Isabel Lopes de Carvalho[4], Ana Pelerito[4], Rita Cordeiro[4], João Paulo Gomes[1,5]

[1]Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [2]Technology and Innovation Unit, Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [3]National Reference Laboratory of Sexually Transmitted Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [4]Emergency Response and Biopreparedness Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [5]Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal;
* These authors contributed equally

The largest monkeypox virus (MPXV) outbreak described to date in non-endemic countries was identified in May 2022. By 13th September 2022, more than 60,000 confirmed MPXV cases had been reported from >100 countries, with Portugal reporting >900 cases so far.
Using shotgun metagenomics and targeted whole genome amplification, we have sequenced >300 MPXV genomes. Sequences were analysed using INSaFLU and Reportree bioinformatics pipelines.
We rapidly sequenced and publicly released the first MPXV genome of the 2022 outbreak by 20th May. This revealed that the outbreak variant belonged to sublineage B.1 of Clade IIb (former West African clade) and that it diverged by ~50 SNPs from 2018-2019 cases that were epidemiologically linked to an endemic country (Nigeria). This pointed to a likely continuous circulation of the 2018-2019 MPXV variant, later leading to the 2022 outbreak. The integration of PT sequences in global phylogeny and identification of clusters unveiled cross-border and local/national transmission chains. Noteworthy, the higher than expected mutation rate and its associated pattern points to a case of evolution driven by host APOBEC3. We found signs of ongoing microevolution (e.g., SNPs, minor variants and gene loss) during human-to-human transmission. Several mutated proteins are known to interact with host immune system, constituting targets for future functional studies on MPVX adaptation.
This study sheds light on MPXV adaptive evolution and indicates that genome sequencing might provide resolution to track the spread of this presumably slow-evolving dsDNA virus. Additionally, it contributed to the definition of the novel international MPXV clade and lineages nomenclature.

Acknowledgements
HERA project (Grant/2021/PHF/23776) supported by the European Commission through the European Centre for Disease Control and Prevention; GenomePT project (POCI-01-0145-FEDER-022184), supported by COMPETE 2020-POCI, Lisboa2020 and CRESC Algarve2020, under the PORTUGAL 2020 Partnership Agreement, through the ERDF, and by FCT; ERINHA-Advance project (funding from the European Union's Horizon 2020 Research and Innovation program); One Health European Joint Programme (EJP).

O7

RNA as a promising molecule to treat a rare neurodegenerative lysosomal storage disorder

Juliana Inês Santos[1,2,3,4], Mariana Gonçalves[1,5], Liliana Matos[1,3,4], Paulo Gaspar[6], Mª João Pires[5], Paula A. Oliveira[5], Mª João Prata[2,7], Mª Francisca Coutinho[1,3,4], Sandra Alves[1,3,4]

[1]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2]Biology Department, Faculty of Sciences, University of Porto, Portugal; [3]Center for the Study of Animal Science, CECA-ICETA, University of Porto, Porto, Portugal; [4]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS); [5]CITAB – Center for the Research and Technology of Agro-Environmental and Biological Sciences, Vila Real, Portugal; [6]Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal; [7]i3S – Health Research and Innovation Institute, University of Porto, Portugal

The RNA molecule is a specific and essential regulator of gene expression.
In our lab, we are addressing the potential of different RNA-based drugs to correct/ameliorate the sub-cellular phenotype of a group of genetic diseases caused by lysosomal dysfunction. Our major efforts are focused on those presenting a predominant neurological phenotype, such as the Sanfilippo syndrome.
Briefly, two major research lines are being pursued: the first is a mutation-specific approach to correct abnormal splicing; the second depends upon selective downregulation of one gene involved in the biosynthesis of the substrate that accumulates in this pathology.
For the splicing correction approach, we are using U1snRNA to restore the splicing defect caused by the *HGSNAT* mutation c.234+1G>A. We demonstrated in vitro that a modified U1snRNA vector designed to improve the definition of *HGSNAT* exon 2 could partially restore its normal splicing (1). Preliminary assessments in mice transiently expressing the human defect have also been performed. Future analysis will rely on AAV-mediated gene transfer techniques. For the substrate reduction approach, we are using siRNAs. By acting over a specific biosynthethic cascade, siRNAs promote an overall decrease of the accumulating substrate. So far, we have already tested this approach in patients' fibroblasts and observed a high inhibition of the target mRNA.
Overall, there are substantial differences between these two approaches but they also face common challenges and show equally promising results.

Acknowledgements
This work is partially supported by the Portuguese Society for Metabolic Disorders (Sociedade Portuguesa de Doenças Metabólicas, SPDM—Bolsa SPDM de apoio à investigação Dr Aguinaldo Cabral 2018 (2019DGH1629/SPDM2018I&D) and 2019 (2020DGH1834)), the Sanfilippo Children's Foundation (SCF Incubator Grant 2019: 2019DGH1656/SCF2019I&D), the MPS Society (2019DGH1642), Center for the Study of Animal Science (CECA; UIDB/00211/2020) and Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS; LA/P/0059/2020).

References
1. Matos *et al*., 2014 (DOI: 10.1186/s13023-014-0180-y).

O8

Up-frameshift 1 (UPF1) internal ribosome entry-site-mediated translation and its importance in colorectal cancer

Adriana Elias[1,2], Rafaela Lacerda[1,2], Juliane Menezes[1,2], Luísa Romão[1,2]

[1]Department of Human Genetics, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, s/n, 1649-016 Lisboa, Portugal; [2]BioISI — Biosystems & Integrative Sciences Institute, University of Lisbon, 1749-016 Lisboa, Portugal

Translation initiation is the limiting step of protein synthesis. In stress conditions, canonical translation is inhibited and the translation of specific transcripts is maintained by alternative mechanisms such as those involving IRES (*internal ribosome entry sites*). UPF1 (*upframeshift 1*) contributes to tumorigenesis in colorectal cancer (CRC). Aiming to understand how the non-canonical translation initiation mechanisms of UPF1 contribute to the progression of CRC, we have shown that its 5' untranslated region (5'UTR) has IRES activity, which is maintained in stress conditions. Accordingly, the analysis of UPF1 endogenous expression in cultivated epithelial cells of the normal human colon mucosa (NCM460), and in cell lines corresponding to different stages of CRC development (Ht29, HCT116 and Sw480), exposed to 1 µM of thapsigargin, which induces endoplasmic reticulum stress and hence inhibits canonical translation, revealed that UPF1 expression is maintained in conditions in which canonical translation is inhibited, suggesting that the IRES-mediated translation allows the maintenance of UPF1 levels in these conditions. *In silico* analyses through Xena platform to measure the variation of mRNA and protein expression among different types of cancer and between normal and carcinogenic colon tissues showed that mRNA and protein expression is higher in CRC tissues than in other cancers and also compared to normal colon tissues, suggesting that expression of UPF1 is higher when UPF1 contributes to tumorigenesis. In conclusion, IRES-mediated translation contributes to the synthesis of UPF1 in CRC tissues, which in turn contributes to the progression of this type of cancer.

Acknowledgements

This project was funded by UID/MULTI/04046/2019 Research Unit grant (to BioISI), PTDC/MED-ONC/32048/2017 and Instituto Nacional de Saúde Doutor Ricardo Jorge.

O9

Toxicity assessment of consumer-relevant nanomaterials in human barrier cells

Joana Pires[1,2,3], Luciana Moreira[1,2,3], João Paulo Teixeira[1,2,3], Sónia Fraga[1,2,3]

[1]Departamento de Saúde Ambiental, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Porto, Portugal; [2]EPIUnit – Instituto de Saúde Pública da Universidade do Porto, Portugal; [3]Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal.

The increasing number of products containing nanomaterials (NM) has raised serious concerns regarding their human safety. This study aimed at evaluating the influence of the chemical composition [Ag, Au, TiO2, SiO2 and graphene oxide (nano_GO)], primary size (10, 30 and 60 nm Ag- and AuNP), crystal structure (TiO2NP rutile/anatase and anatase) and surface coating (citrate and PEGylated AuNP) for the potential induced toxic effects in human intestinal and placental epithelial cells. Toxicity was assessed by determining alterations in cell morphology, metabolic activity, plasma membrane integrity, intracellular ROS and ATP levels, and DNA integrity at 24 h after exposure to the NM. The toxicity profile of the tested NM was similar in both human barrier models, however placental epithelial cells were more sensitive than intestinal epithelial cells. Overall, NM can be ranked for cytotoxicity as AgNP > nano_GO > AuNP ~ TiO2 NP ~ SiO2NP, being the effects more visible at higher concentrations. The size's role was more evident for Ag- than for AuNP, with the smaller NP inducing more cytotoxic effects. PEG capping was effective for preventing AuNP cytotoxicity. Exposure to AgNP and nano_GO significantly increased ROS levels suggesting that oxidative stress is a possible mechanism underlying their cytotoxicity. All tested NM significantly increased intracellular ATP levels, except 10 nm AuNP. Interestingly, a greater cytotoxic potential did not necessarily translate into a greater genotoxic potential as only AgNP (positive) and anatase TiO2NP (equivocal) induced DNA damage. Our findings alert for the potential risks associated with human barriers exposure to NM.

Acknowledgements
Study carried out under the project NanoBioBarriers (PTDC/MED-TOX/31162/2017) funded by the Operational Program for Competitiveness and Internationalization (POCI) through European Regional Development Funds (FEDER/FNR) and through national funds by the Portuguese Foundation for Science and Technology (FCT). S. Fraga thanks FCT for funding through program DL 57/2016 – Norma transitória (Ref. DL-57/INSA-06/2018).

O10

Pro-Inflammatory Cytokines induce changes in expression of Tumour-Related Splice Variant RAC1B in Polarized Colorectal Cells

Joana F S Pereira[1,2], Cláudia Bessa[1,2], Vânia Gonçalves[1,2], Paulo Matos[1,2], Peter Jordan[1,2]

[1]Department of Human Genetics , National Institute of Health 'Dr. Ricardo Jorge', Lisbon, Portugal
[2]BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Portugal

An inflammatory microenvironment is a tumour-promoting condition that provides survival signals to which cancer cells respond with gene expression changes. One example is the alternative splicing variant Rat Sarcoma Viral Oncogene Homolog (Ras)-Related C3 Botulinum Toxin Substrate 1 (RAC1)B, which we previously identified in a subset of V-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF)-mutated colorectal tumours. RAC1B was also increased in samples from inflammatory bowel disease patients or in an acute colitis mouse model. Here, we used an epithelial-like layer of polarized Caco-2 or T84 colorectal cancer (CRC) cells in co-culture with fibroblasts, monocytes or macrophages and analysed the effect on RAC1B expression in the CRC cells by RT-PCR, Western blot and confocal fluorescence microscopy. We found that the presence of cancer-associated fibroblasts and M1 macrophages induced the most significant increase in RAC1B levels in the polarized CRC cells, accompanied by a progressive loss of epithelial organization. Under these conditions, we identified interleukin (IL)-6 as the main trigger for the increase in RAC1B levels, associated with Signal Transducer and Activator of Transcription (STAT)3 activation. IL-6 neutraliza- tion by a specific antibody abrogated both RAC1B overexpression and STAT3 phosphorylation in polarized CRC cells. Our data identify that pro-inflammatory extracellular signals from stromal cells can trigger the overexpression of tumour related RAC1B in polarized CRC cells. The results will help to understand the tumourpromoting effect of inflammation and identify novel therapeutic strategies.

Acknowledgements
Grants UID/MULTI/04046/2019, SFRH/BD/109162/2015 and PTDC/BIA-MOL/28386/2017 from FCT.

Posters

P1

Ancestry of the major long-range regulatory site of the α-globin genes in the Portuguese population with the common 3.7kb α-thalassemia deletion

Rita Pena[1], Pedro Lopes[1], Gisela Gaspar[2], Armandina Miranda[2], Paula Faustino[1,3]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [2]Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [3]Instituto de Saúde Ambiental, Faculdade de Medicina, Lisboa; Portugal.

The α-major regulatory element (known as HS-40) has a crucial role in the long-range regulation of the α-globin gene expression. This element is genetically polymorphic and six haplotypes (A to F) have been identified in different populations, with haplotype D almost exclusively found in African populations. This study aimed toidentify the HS-40 haplotype associated with the common 3.7kb α-thalassemia deletion (-α3.7del) in the Portuguese population, and investigate its ancestry. We searched for the -α3.7del in 111 selected Portuguese individuals by Gap-PCR. In addition, a DNA fragment containing the HS-40 was amplified by PCR and Sanger sequenced. Statistical analysis was performed using R software.
Fifty individuals have the wild-type α-globin genotype (group I), 34 are heterozygous for the -α3.7del (group II) and 27 are homozygous (group III). Regarding the HS-40, four haplotypes were found (A to D). The distribution of HS-40 haplotypes and genotypes are significantly different between groups with and without the -α3.7del ($p<0.001$), being haplotype D and genotype AD the most prevalent in group III. Furthermore, multiple correspondence analysis revealed that individuals without the -α3.7del are grouped with other European populations, while samples with the -α3.7del are split from these and found more related to the African population.
This study revealed for the first time an association of a specific HS-40 haplotype with the -α3.7del in the Portuguese population, and its likely African ancestry. These results may have a clinical importance as in vitro analysis of haplotype D showed a descrease in its enhancer activity on α-globin genes.

Acknowledgements
We would like to thank the Technology and Innovation Unit of DGH/INSA for Sanger sequencing analyses.

P2

iPSCs and NGS as the basis for a Tay Sachs disease variant B1 cellular model

Diogo Ribeiro[1,2,3,4,] Ana Joana Duarte[1,2,3,4], Luciana Moreira[3,4], Renato Santos[1], Marisa Encarnação[1,3,4], Lisbeth Silva[1], Sandra Alves[1,3,4], Olga Amaral[1,3,4]

[1]Department of Human Genetics, Unit of Research & Development, National Health Institute Ricardo Jorge, Porto, Portugal; [2]ICBAS, University of Porto, Porto; [3]CECA, ICETA, University of Porto, Porto; [4]AL4AnimalS, Portugal.

Tay Sachs disease variant B1 (TSD B1; OMIM 272800) is a neurodegenerative lysosomal storage disease (LSD) which, although rare, it is the most frequent form of TSD in Portugal. The availability of disease-relevant cell types derived from induced pluripotent stem cells (iPSCs) provides a model for studying the pathogenic mechanisms and, eventually, test therapeutic approaches for TSD B1 patients. The main objectives of this project are: establish iPSCs from patient fibroblasts,generate a neuronal TSD B1 specific cellular model and implement the genetic profiling by Next Generation Sequencing (NGS) to examine potential changes in the manipulated cells. First, iPSCs from a control fibroblast cell line and from TSD B1 fibroblasts were obtained by using a non-integrative approach with episomal vectors, the control was further differentiated into neural progenitor cells (NPCs).
Those results as well as the NGS results from the donor cells are presented in this work.
By using a customized NGS panel, we obtained results of the cells in a "naïve" state to later compare with TSD B1 iPSCs, TSD B1 NPCs and NPCs obtained from control iPSCs.
The iPSC reprogramming was accomplished and differentiation into NPCs was also achieved. Perseverance is crucial in this type of highly meticulous work where minor things, such as lack of liquid nitrogen or a mycoplasma infection, may force a start over.

Acknowledgements
We thank the collaboration of INSA's DGH and the support from FCT PTDC/BIM-MEC/4762/2014 in personal grants and consumables.

P3

iPSCs-derived Cardiomyocytes and Future Perspectives

Ana Joana Duarte [1,2,3,4], Luciana Moreira [1,2,3], Diogo Ribeiro [1,2,3], Olga Amaral [1,2,3]

[1]Departamento de Genética Humana – UID Porto, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA, IP); [2]Centro de Estudos de Ciência Animal (CECA), Universidade do Porto (UP); [3]Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS); [4]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP)

Fabry disease (FD) is one of the commonest Lysosomal Storage Disorders (LSDs) and is caused by mutations in the alpha-galactosidase A gene (GLA) from which results a deficient activity of the lysosomal hydrolase alphagalactosidase A (α-Gal A). This deficiency leads to progressive multisystemic accumulation of glycolipids, namely, globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), in plasma and in a wide range of cells, particularly in the relevant cells affected by the disease like cardiomyocytes.

One of the aims of this work was to differentiate induced pluripotent stem cells (iPSCs) reprogrammed from FD patients' fibroblasts into cardiomyocytes (one of the celltargets of FD), is entirely accomplished.

For this purpose, we reprogrammed FD patients' cells and a normal control cell line using the non-integrative episomal vectors (Epi5). After achieving the iPSCs state, the cells were submitted to differentiation using specific cardiomyocytes effectors, and we obtained functional iPSC-cardiomyocytes (iPSC-CMs) that express relevant physiological markers and present contractility. The resulting iPSC-CMs will be analyzed against the initial FD fibroblast to see if the disease features are replicable in the new cell lines.

Another aim is to correct the missense mutation present in this particular cell line (p.W287X) through Prime Editing (PE), a CRISPR-Cas9 derived method. One of the major advantages of this method is that PE mitigates the need of double strand breaks (DSB) repair machinery, which is notoriously error prone, so off-target effects are almost undetectable in comparison to Cas9 DSB-dependent repair system. Presently, the strategy for PE experiments is ongoing.

Funding: Fundação para a Ciência e a Tecnologia: PTDC/BIM-MEC/4762/2014 and SFRH/BD/118009/2016.

P4

Molecular study of MODY: 11 years of personalised medicine

Margarida Vaz[1], Gisela Gaspar[1], Ana Agapito[2], Ana Carolina Neves[3], Ana Paula Bogalho[4], Bruno Almeida[3], Carla Pereira[5], Fernando Fonseca[2], Goreti Lobarinhas[6], Henrique Vara Luiz[7], João Sequeira Duarte[8], Maria de Lurdes Sampaio[5], Paulo Dario[1], Mafalda Bourbon[1]

[1]Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]Serviço de Endocrinologia. Hospital Curry Cabral, Centro Hospitalar Universitário Lisboa Central, Lisboa, Portugal; [3]Associação Protetora dos Diabéticos de Portugal, Lisboa, Portugal; [4]Serviço de Endocrinologia, Diabetes e Metabolismo. Centro Hospitalar Universitário Lisboa Central, Lisboa, Portugal; [5]Unidade de Endocrinologia Pediátrica. Serviço de Pediatria Médica. Departamento de Pediatria. Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal; [6]Serviço de Pediatria, Hospital Santa Maria Maior, Barcelos, Portugal; [7]Serviço de Endocrinologia e Diabetes, Hospital Garcia de Orta, Almada, Portugal; [8]Serviço de Endocrinologia, Hospital Egas Moniz, Lisboa, Portugal.

Background and objective(s)

MODY diabetes is a monogenic condition caused by a genetic variant in one of 14 genes associated with this disease. This condition is often misdiagnosed as type 1 or type 2 diabetes, which can result in inadequate disease management. Genetic testing is the only method that provides the correct diagnosis to patients and their clinicians. Here, we present the latest results of our 11-year project.

Materials and methods

Blood samples were collected from 112 index cases and their relatives, from which DNA was extracted for PCR amplification and DNA sequencing. The promoter region, coding sequences and adjacent intronic regions of GCK, HNF1A, HNF1B and HNF4A genes were screened for genetic variants. This was followed by in sílico analysis of the identified variants and they were classified according to ACMG recommendations. Large rearrangements studies were done by MLPA.

Results

A pathogenic variant was identified in 32 index patients and, through cascade screening, 24 relatives were also diagnosed with MODY. It was not possible to identify a genetic aetiology for 41 index patients.

Discussion and conclusion(s)

Although we didn´t find a pathogenic variant in 36% of the index patients, we suspect that some of them may have alterations in the rarer genes and we hope to be able to study them with a NGS MODY panel that we are validating in the department. There are still many challenges that we want to overcome so that the concept of personalized medicine becomes an accessible reality for MODY patients in Portugal.

P5

Long read nanopore sequencing: new approach to the methylation profile of the human genome

Catarina Silva[1,2], Miguel Machado[1], José Ferrão[1], Sebastião Rodrigues[2], Luís Vieira[1,2]

[1]Unidade de Tecnologia e Inovação, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal; [2]Centro de Toxicogenómica e Saúde Humana (ToxOmics), NOVA Medical School|Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Campo dos Mártires da Pátria 130, 1169-056 Lisboa, Portugal.

Methylation of cytosine in CpG dinucleotides [5'-methylcytosine (5'-MC)] plays an important role in human gene regulation. Although different methodologies have been used to characterize 5'-MC in human cells, these usually require prior modification/selection of DNA sequences. These manipulations of DNA can result in an incorrect assessment of the methylation status of CpG dinucleotides due to technical biases. Here we describe the use of long-read nanopore sequencing (NS) of native DNA to characterize 5'-MC in the whole human genome sequence, as well as a benchmarking comparison with short-read sequencing based-methodologies (DREAM & RRBS) and 450k methylation array.

Genomic DNA was obtained from the human myeloid cell line HEL and sequenced in a MinION device (Oxford Nanopore Technologies, ONT), following a rapid library preparation protocol (ONT). Reads were processed using the nanopype pipeline. R scripts were used to compare methylation frequencies (MF) between NS, 450k microarray, DREAM and RRBS for HEL.

Using NS we obtained MF for virtually all CpGs present in the Hg38 human reference sequence at ~10X average depth of coverage. 5'-MC MF were highly correlated with those obtained for DREAM, RRBS and 450k microarray in the whole genome sequence. Moreover, NS revealed a faithful representation of the CpG profiles in various genomic contexts, including CpG islands and transcriptional start sites.

NS provides unbiased whole genome methylation calling without any DNA modification, thus minimizing methodological biases. In conclusion, low coverage depth nanopore sequencing is a fast, robust and sensitive approach to the study of methylation in the human genome.

Acknowledgements

This work is a result of the GenomePT project (POCI-01- 0145-FEDER-022184), supported by COMPETE 2020 – Operational Programme for Competitiveness and Internationalisation (POCI), Lisboa Portugal Regional Operational Programme (Lisboa2020), Algarve Portugal Regional Operational Programme (CRESC Algarve2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and by Fundação para a Ciência e a Tecnologia (FCT). This work was also supported by Fundos FEDER through the Programa Operacional Factores de Competitividade – COMPETE and by Fundos Nacionais through the FCT within the scope of the project UID/BIM/00009/2019 (Centre for Toxicogenomics and Human Health-ToxOmics).

Trabalho publicado em Silva *et al.*(2022): Whole human genome 5'-mC methylation analysis using long read nanopore sequencing, Epigenetics, DOI: 10.1080/15592294.2022.2097473.

P6

The disease modelling value of baby teeth: A new way to unlock knowledge about a special group of genetic disorders

Sofia Carvalho[1,2], Juliana Inês Santos[1,3,4,5], Luciana Moreira[1,3,4], Paulo Gaspar[6], Mariana Gonçalves[1], Liliana Matos[1,3,4], Marisa Encarnação[1,3,4], Diogo Ribeiro[1,3,4], Ana Joana Duarte[1,3,4], Maria João Prata[6], Maria Francisca Coutinho[1,3,4], Sandra Alves[1,2]

[1]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2]Faculty of Pharmacy, University of Coimbra, Portugal; [3]Center for the Study of Animal Science, CECA-ICETA, University of Porto, Porto, Portugal; [4]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS); [5]Biology department, Faculty of Sciences, University of Porto, Portugal; [6]Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal; [7]i3S – Health research and innovation institute, University of Porto, Portugal

Mucopolysaccharidoses (MPS), are a group of genetic, metabolic, and rare diseases investigated since the early years of the 20th century. One of the first steps to collect information about the underlying mechanisms of those disorders is the development and analysis of in vitro models. Furthermore, those models provide an appropriate platform for the evaluation of future therapeutics. Among all the possible disease cell models, patient-derived ones are those which allow us to get better disease insights. However, finding the best cell type that recapitulates diseaserelevant features is not always easy: two systems largely involved in MPS pathology are the brain and the musculoskeletal ones, which reflects an issue once both are hard to access.

Here, our main goal is to establish an innovative non-invasive method to generate disease-relevant cell models from stem cells from deciduous (baby) teeth (SHED), which may then be differentiated into our MPS-target cell lines.

So far, we have already implemented and optimized the protocol for collection, isolation, establishment and cryopreservation of those stem cells. Then, our rationale is simple: for those obtained from MPS patients suffering from multisystemic disease with marked musculoskeletal alterations, we are using a chondrogenesis differentiation protocol. For those derived from patients with neurological pathology, we will establish mixed neuronal/glial cultures. As soon as we can get the SHED-derived differentiated cells, various cellular and molecular processes from our target disorders may be unveiled and used as a target for possible future therapeutics.

Acknowledgements

This work is partially supported by the Portuguese Society for Metabolic Disorders (SPDM - Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018;2019DGH1629/SPDM2018I&D), Sanfilippo Children's Foundation (2019DGH1656/SCF2019I&D) and FCT (EXPL/BTM-SAL/0659/2021).

P7

An Antisense Oligonucleotide based therapy for a rare disease: *in vitro* and *in vivo* studies

Mariana Gonçalves[a,d*], Liliana Matos[a,b*], Juliana I. Santos[a,b,c], Maria Francisca Coutinho[a,b], Maria João Prata[c,e], Maria João Pires[d], Paula Oliveira[d], Sandra Alves[a,b]

[a]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [b]Center for the Study of Animal Science, CECA-ICETA, University of Porto; [c]Biology department, Faculty of Sciences, University of Porto, Portugal; [d]Center for the research and technology of agro-environmental and biological sciences (CITAB/UTAD); [e]i3S – Health research and innovation institute, University of Porto, Portugal. *These authors contributed equally to this work.

Mucolipidosis II (MLII) is caused by the deficiency of the enzyme GlcNAc-1-phosphotransferase. This is responsible for the mannose-6-phosphate marker addition to lysosomal enzymes. From the several MLII mutations, the deletion of 2 nucleotides from *GNPTAB* exon 19 (c.3503_3504del) is the most frequent, making it a good target for a mutation specific therapy.

In a previous study in fibroblasts from MLII patients, Antisense Oligonucleotides (ASOs) were used to skip exon 19 from the *GNPTAB* pre-mRNA, resulting resulting in the production of an in-frame mRNA[1].

Currently, our aim is to evaluate the therapeutic potential of this approach, both *in vitro* in C57BL/6 fibroblasts and *in vivo* in C57BL/6 mice.

As for the *in vitro* expression assays, the C57BL/6 fibroblasts were transfected with ASOs concentrations ranging from 10nM to 600nM. After 24h or 48h of incubation, cells were collected and cDNA analysis revealed a full-length transcript but also another one of lower molecular weight compatible with exon-skipping.

In *in vivo* studies, 18 animals were used, divided into 6 groups: groups 1 and 4 were injected with saline solution, groups 2 and 5 were injected with the ASO at 25 mg/kg and groups 3 and 6 were injected with the ASO at 50 mg/kg. After 4 or 7-days post-treatment, the animals were sacrificed and the organs collected. After molecular analysis the exon 19 skipping was not observed.

So, we can theorize that the doses administered were not sufficient to elicit a response or the ASO might have had a high clearance rate. These are preliminary data, so in the near future more experiments will be done.

References

1. Matos L, Vilela R, Rocha M, *et al*. Hum Gene Ther, 2020, 31(13-14):775-783.

P8

Portuguese Familial Hypercholesterolemia Study as the basis of APOB Variants Database

Maria Ferreira[1]; Joana Rita Chora[1,2]; Ana Margarida Medeiros[1,2]; Mafalda Bourbon[1,2] and Ana Catarina Alves[1,2]

[1]Grupo de Investigação Cardiovascular, Unidade I&D, Departamento de Promoção da Saúde e Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

Background and objectives: Familial hypercholesterolemia (FH) is an autosomal semi dominant disorder of lipid metabolism with increased cardiovascular risk. The genetic diagnosis is usually based on the analysis of *LDLR*, *APOB*, and *PCSK9* genes. *APOB* variants are responsible for about 5%-10% of FH cases and only recently the whole gene has been sequenced due to next generation sequencing, increasing the variant spectrum of *APOB*. To facilitate the analysis of these variants, we created a database including all *APOB* variants found up to date in the Portuguese FH Study.

Materials and methods: We verified in the ClinVar and PubMed repositories if the variants had already been reported. When available, we collected information about the minor allele frequency on gnomAD and the functional characterization of the variants. All variants were classified by American Colleges of Medical Genetics guidelines.

Results: We have identified 164 variants, of which 101 are missense, 33 synonymous, 3 deletions, 3 frameshifts, and 1 nonsense. Only 12 variants have functional studies, half of them performed by our group, including one deletion. From all, 3 variants were classified as pathogenic/likely pathogenic and 29 as benign/likely benign. The remaining variants are variants of uncertain significance, because of poor evidence and knowledge about them, including lack of functional characterization. Our group is currently performing functional studies on variants in the *APOB* gene.

Discussion and conclusions: These studies are important to characterize the variants, including frameshift variants, usually associated with hypocholesterolemia. We aim to increase our database with variants found worldwide to increase the scientific knowledge of these variants and improve FH diagnosis.

Personalized Medicine in Familial Hypercholesterolemia – diagnosis, stratification of cardiovascular disease risk and lipid therapy management

Joana Rita Chora[1,2] and Mafalda Bourbon[1,2]

[1]Grupo de Investigação Cardiovascular, Unidade I&D, Departamento de Promoção da Saúde e Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

Familial hypercholesterolemia (FH) is a common lipid metabolism disorder, caused by pathogenic variants in *LDLR*, *APOB* and *PCSK9*. Its characteristic life-long elevated cholesterol levels make it a high cardiovascular disease (CVD) risk condition. Treatment of FH is made primarily with statins, but there is a wide variability in response, that could be caused by type of FH variant and/or variants in statin pharmacogenetics' genes. This PhD aims at producing evidence to improve FH genetic diagnosis, treatment and CVD prevention.
A database of all published FH-associated variants was constructed to ensure quick access to data for genetic diagnosis. From this groundwork, an internationally approved guideline for *LDLR* variant pathogenicity classification was developed by integratingan international consortium. With this FH experts' network, FH variant data publicly available at a reference repository of clinical genetic information increased 10-fold. Specific adaptations for *APOB* and *PCSK9* variant classification were also proposed.
To improve CVD prevention, treatment patterns were studied, individual risk factors and overall risk were analyzed in FH patients and in the Portuguese general population. Results showed that FH patients had four times more CVD events, and although more likely to be medicated, they are less likely to meet recommended target lipid values.
Finally, a preliminary study on statin pharmacogenetics yielded some promising results that, if validated in larger studies, could explain variability of response to statins.
An accurate diagnosis of FH, precise individual CVD risk stratification and individually adjusted therapy to maximize CVD prevention are the basis of Personalized Medicine in FH.

Acknowledgements
J.R.C. acknowledges her PhD fellowship (SFRH/BD/108503/2015) funded by FCT - Fundação para a Ciência e Tecnologia do Ministério da Ciência, Tecnologia e Ensino Superior.

P10

Genetic diagnosis of Familial hypertriglyceridemia

Beatriz Miranda[1,2], Ana Catarina Alves[1,2], Mafalda Bourbon[1,2]

[1]Grupo de Investigação Cardiovascular, Unidade I&D, Departamento de Promoção da Saúde e Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

Background and objective(s): Hypertriglyceridemia is clinically defined by abnormal triglyceride (TG) concentrations (>185mg/dL). Particulalry in the intermediate (500-800mg/dL) or severe (>800mg/dL) range, this condition can be genetic (typically autosomal recessive) and is associated to serious hepatic, pancreatic and atherosclerotic complications. This study aimed to identify the molecular cause of hypertriglyceridemia in 54 Portuguese patients with TG>500mg/dL.

Materials and methods: The molecular analysis of 6 genes associated to genetic hypertriglyceridemia (*LPL/APOA5/APOC2/LMF1/GPIHBP1/GPD1*) was performed by PCR and Sanger Sequencing or more recently using a Next Generation Sequencing gene panel.

Results: A cause of disease was identified in 23 individuals. Seven patients were diagnosed with Familial chylomicronemia syndrome (FCS): 2 are compound heterozygous (*LPL* gene) and 5 are true homozygous (3 for the *LPL*, 1 for the *APOC2* and 1 for the *GPIHBP1* genes). Fifteen patients diagnosed with Multifactorial chylomicronemia presented heterozygous variants in the *LPL* (8), *APOA5* (6) and *LMF1* (1) genes. One patient (true homozygous for a *GPD1* gene variant) was diagnosed with Transient infantile hypertriglyceridemia. Four variants with uncertain significance were detected in the *LPL/APOA5/LMF1* genes in 4 individuals (one individual can be a compound heterozygous). Twenty-seven individuals will remain understudy since no variants were found in the analysed genes.

Discussion and conclusion(s): Despite identifying the cause of disease in 23/54 patients (43%), the positive rate would increase considering the 4 VUS, demonstrating the importance of investigating the role of heterozygosities in FCS genes. The identification of patients with familial hypertriglyceridemia is fundamental for the early prevention of disease complications and for a personalized treatment based on the pathway affected.

P11

A role for gene-environment interactions in Autism Spectrum Disorder is supported by variants in genes regulating the effects of exposure to xenobiotics

João Xavier Santos[1,2], Célia Rasga[1,2], Ana Rita Marques[1,2], Hugo Martiniano[1,2], Muhammad Asif[1,2], Joana Vilela[1,2], Guiomar Oliveira[3,4], Lisete Sousa[5], Ana Nunes[2,6], Astrid Vicente[1,2]

[1]Departamento de Promoção da Saúde e Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal; [2]BioISI–Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisbon, Portugal; [3]Unidade de Neurodesenvolvimento e Autismo, Serviço do Centro de Desenvolvimento da Criança, Centro de Investigação e Formação Clínica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [4]Faculty of Medicine, University Clinic of Pediatrics and Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Coimbra, Portugal; [5]Departamento de Estatística e Investigação Operacional e Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; [6]Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

Background and objective(s): Heritability estimates support a role for gene-environment interactions in the etiology of Autism Spectrum Disorder (ASD). Detoxification pathways and physiological permeability barriers (e.g., blood-brain barrier, placenta and respiratory airways) regulate the effects of early life exposure to xenobiotics, when the immature brain is extremely vulnerable.

Our objective was to identify predicted damaging variants in detoxification and barrier genes (XenoReg genes), in subjects with ASD, and to understand their interaction patterns with ubiquitous xenobiotics previously implicated in the disorder.

Materials and Methods: Large ASD datasets were inspected for predicted damaging Single Nucleotide Variants (N=2674) or Copy Number Variants (N=3570) in 519 XenoReg genes. We queried the Comparative Toxicogenomics Database (CTD) to identify gene-environment interaction pairs.

Results: We prioritized 77 XenoReg genes with high evidence for a role in ASD, according to pre-specified criteria. These include 47 genes encoding detoxification enzymes and 30 genes encoding barrier proteins, among which 15 are known ASD candidates. The CTD query revealed 397 interaction pairs between these genes and 80% (48/60) of the xenobiotics. The top interacting genes and xenobiotics were, CYP1A2, ABCB1, ABCG2, GSTM1, and CYP2D6 and benzo-(a)-pyrene, valproic acid, bisphenol A, particulate matter, methylmercury, and perfluorinated compounds.

Discussion and conclusion(s): Individuals carrying predicted damaging variants in high evidence XenoReg genes may be particularly susceptible to early life exposure to xenobiotics, which elicit neuropathological mechanisms, such as epigenetic changes, oxidative stress, neuroinflammation and endocrine disruption. As exposure to xenobiotics may be mitigated, this work provides new perspectives to personalized prevention in ASD.

Acknowledgements

This work was supported by the Fundação para a Ciência e a Tecnologia (FCT), through funding to the project "Gene-environment interactions in Autism Spectrum Disorder". JXS, ARM, MA, and JV are fellows of the BioSys Ph.D Program and awardees of scholarships funded by FCT. AGP data was collected from patients genotyped in the context of Autism Genome Project (AGP), funded by NIMH, HRB, MRC, Autism Speaks, Hilibrand Foundation, Genome Canada, OGI, and CIHR. ASC data was collected from patients genotyped in the context of Autism Sequencing Consortium supported by NIH grants U01MH100233, U01MH100209, U01MH100229, and U01MH100239.

P12

Unraveling the hurdles of a large COVID-19 epidemiological investigation by viral genomics

Regina Sá[a], Joana Isidro[b], Vítor Borges[b], Sílvia Duarte[c], Luís Vieira[c], João P Gomes[b], Sofia Tedim[d], Judite Matias[a], Andreia Leite[e,f]

[a]Public Health Unit of the Baixo Vouga Health Center Grouping, Regional Health Administration of the Center Portugal (ARSC), Aveiro, Portugal; [b]Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [c]Innovation and Technology Unit, Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [d]Department of Mathematics, University of Aveiro (UA), Aveiro, Portugal; [e]NOVA National School of Public Health, Public Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal; [f]Comprehensive Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal

COVID-19 local outbreak response relies on subjective information to reconstruct transmission chains. We assessed the concordance between epidemiologically linked cases and viral genetic profiles, in the Baixo Vouga Region (Portugal), from March to June 2020.
A total of 1925 COVID-19 cases were identified, with 1143 being assigned to 154 epiclusters. Viral genomic data was available for 128 cases. Public health authorities identified two large epiclusters (280 and 101 cases each) with a central role on the spread of the disease. Still, the genomic data revealed that each epicluster included two distinct SARS-CoV-2 genetic profiles and thus more than one transmission network. We were able to show that the initial transmission dynamics reconstruction was most likely accurate, but the increasing dimension of the epiclusters and its extension to densely populated settings (healthcare and nursing home settings) triggered the misidentification of links. Genomics was also key to resolve some sporadic cases and misidentified direction of transmission. The epidemiological investigation showed a sensitivity of 79.55% (95%CI: 70.42%, 86.40%) to detect transmission chains.
This study contributes to the understanding of the hurdles and caveats associated with the epidemiological investigation of hundreds of community cases in the context of a massive outbreak caused by a highly transmissible and new respiratory virus.

Acknowledgements
This study did not receive any funding.

P13

Association between area-level walkability and glycosylated haemoglobin: a Portuguese population-based study

Regina Sá[a], Rita Roquette[b], Andrea Rebecchi[c], Judite Matias[a], Jorge Rocha[d], Maddalena Buffoli[c], Stefano Capolongo[c], Ana Isabel Ribeiro[e,f,g], Baltazar Nunes[b,h,i], Carlos Dias[b,h,i], Mafalda Sousa-Uva[b,h,i]

[a]Public Health Unit, Baixo Vouga Primary Care Cluster, Aveiro, Portugal; [b]Department of Epidemiology, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal; [c]Design&Health Lab, Department of Architecture, Built environment and Construction Engineering, Polytechnic University of Milan, Italy; [d]Institute of Geography and Territorial Planning, University of Lisbon and Associated Laboratory TERRA, Lisbon, Portugal; [e]EPIUnit, Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal; [f]Departamento de Ciências da Saúde Pública e Forenses e Educação Médica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal; [g]Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal; [h]CISP – Public Health Research Center, NOVA National School of Public Health, Lisbon, Portugal; [i]Comprehensive Health Research Center (CHRC), Lisbon, Portugal

Diabetes is responsible for a high burden of disease but there is a great potential for prevention. Physical inactivity is one of the major risk factors for type 2 diabetes that may be tackled by increasing area-level walkability, but there are still little population-based studies exploring the association between area-level walkability with objective measures of diabetes. The aim of this study is to estimate the association between area-level walkability and individual levels of glycosylated haemoglobin, in the Portuguese adult population.

Area-level data required to estimate walkability was obtained from 2011 census and an updated street map. The walkability index was composed by residential density, land-use mix and street connectivity. Individual-level data was obtained from The National Health Examination Survey (INSEF) 2015, a population-based survey representative of Portuguese adult population. We used gamma regression to estimate associations.

The regression coefficients showed that living in medium walkable areas reduced the average glycosylated haemoglobin (0.903,95%CI:0.819–0.995), when compared to least walkable areas. The association was less clear for the third tertile of walkability. Our findings suggest a non-linear protective effect of walkability on glycosylated haemoglobin. These findings could have important policy implications by highlighting considering walkability in urban planning, with the goal of preventing diabetes and promoting health.

Acknowledgements

Ana Isabel Ribeiro was supported by National Funds through FCT, under the programme of Stimulus of Scientific Employment–Individual Support within the contract CEECIND/02386/2018. This study was also funded by FEDER through the Operational Programme Competitiveness and Internationalization and national funding from the Foundation for Science and Technology – FCT (Portuguese Ministry of Science, Technology and Higher Education) under the Unidade de Investigação em Epidemiologia - Instituto de Saúde Pública da Universidade do Porto (EPIUnit) (UIDB/04750/2020).

P14

Healthcare professionals' psychological distress, risk and protective mental health factors after two years of COVID-19 pandemic in Portugal

Alexandra Costa[1,2], Teresa Caldas de Almeida[1], Mónica Fialho[3], Célia Rasga[1], Hugo Martiniano[1], Osvaldo Santos[3,4], Ana Virgolino[3], Astrid Vicente[1], Maria João Heitor[3,5,6,7]

[1]Departamento de Promoção da Saúde e Prevenção de Doenças Não-Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]Instituto Superior de Ciências Sociais e Políticas, Universidade de Lisboa; [3]Environmental Health Behaviour Lab, Instituto de Saúde Ambiental, Faculdade de Medicina, Universidade de Lisboa, Portugal; [4]Unbreakable Idea Research, Lda., Portugal; [5]Departamento de Psiquiatria e Saúde Mental, Hospital Beatriz Ângelo, Loures, Portugal; [6]Sociedade Portuguesa de Psiquiatria e Saúde Mental, Portugal; [7]Faculdade de Medicina, Universidade Católica Portuguesa, Portugal

Background and objective: The COVID-19 pandemic increased psychosocial riskfactors among healthcare professionals (HCP). The main objective was to characterize Portuguese HCP's mental health (MH) outcomes, estimating the percentage of symptoms of anxiety, depression, post-traumatic stress disorder (PTSD) and burnout, and identifying risk and protective factors.

Materials and methods: A cross-sectional online survey was conducted in 2020 (T0) and repeated in 2021 (T1). Sociodemographic and occupational variables, protection behaviours and pandemic-context data were collected from a non-probabilistic sample of HCP in Portugal. MH outcomes were assessed using instruments with sound psychometric properties. Risk and protective factors were assessed through simple and multiple logistic regression models.

Results: Overall, 2027 participants answered the survey in T0; 1843 in T1. The percentage of moderate to severe symptoms decreased from 2020 to 2021: 26.2% and 23.4% for anxiety (T0 and T1, respectively; p = 0.032), 25.3% and 23.8% for depression, 22.8% and 19.1% for PTSD (p = 0.003), and 29.8% to 29.7% for burnout. Being a woman, working in a COVID-19 treatment-frontline position and having difficulties in work-life balance increased the odds of psychological distress. High resilience, perception of adequate social/family support, and keeping hobbies/lifestyle-routines were found as protective factors.

Discussion and conclusions: The results show a higher percentage of psychological distress symptoms among HCP's when comparing with previous data for the Portuguese population. These findings support further monitoring on the HCP's mental health. Psychological screening and surveillance is of paramount importance in peri- and post-pandemic period.

Acknowledgements: This work was supported by the Fundação para a Ciência e a Tecnologia (FCT) during 2020, the first phase of the project, under the RESEARCH4COVID19 programme.

P15

Association between grip strength and the risk of heart diseases among European middle-aged and older adults

Miguel Peralta[1,2], Carlos Matias Dias[3,4], Adilson Marques[1,2], Duarte Henriques-Neto[5,6], Mafalda Sousa-Uva[3,4]

[1]CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Lisbon, Portugal; [2]ISAMB, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; [3]CISP, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Lisbon, Portugal; [4]Departamento de Epidemiologia, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal; [5]Research Center in Sports Sciences, Health Sciences and Human Development, Maia University, Maia, Portugal; [6]School of Higher Education, Polytechnic Institute of Porto, Porto, Portugal

Background and objective(s): The association between grip strength and heart diseases incidence has been little explored. The aim of this study is to analyse the longitudinal relationship between grip strength and the diagnosis of heart diseases in European middle-aged and older adults.

Material and methods: A prospective cohort study was conducted using data from the Survey of Health, Ageing and Retirement in Europe (2004-2017). Participants were 20829 middle-aged and older adults from 12 countries. Grip strenght was measured by a dynamometer and heart diseases diagnosis was self-reported. Incidence rate of heart diseases was calculated and a Cox proportional hazard regression was performed.

Results: Heart diseases incidence decreased from 930 per 100 000 person-years in the lowest quartile to 380 per 100 000 person-years in the highest grip strength quartile. During the 13 years of follow-up, compared to being in the lowest grip strength quartile, being in the highest quartile decreased the hazard of being diagnosed with a heart disease in 36% (95% confidence interval [CI]: 0.53, 0.78) for the whole sample, 35% (95% CI: 0.51, 0.84) for men and 46% (95% CI: 0.40, 0.73) for women.

Discussion and conclusion(s): Grip strength seems to be inversely associated with the incidence of heart diseases among European middle-aged and older adults. Scientific evidence has highlighted the potential role of grip strength as a risk stratifying measure for heart diseases, suggesting its potential to be included in the cardiovascular risk scores used in primary care. However, further research is still needed to clarify it.

Acknowledgements

This study received no funding, and the authors declare no conflicts of interest. The SHARE project funding over the years is available at http://www.shareproject.org/organisation/funding.html.

P16

Use of quasi-experimental studies to evaluate causal effects of public health interventions in Portugal: a scoping review protocol

Andreia Leite[1,2,3], Irina Kislaya[1,2,3], Ausenda Machado[1,2,3], Pedro Aguiar[2,3], Baltazar Nunes[1,2,3], Carlos Dias[1,2,3]

[1]Departamento de Epidemiologia, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal; [2]NOVA National School of Public Health, Public Health Research Center, Universidade NOVA de Lisboa, Portugal; [3]Comprehensive Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal

Evaluating causal effects of public health interventions using traditional randomized controlled trials might not be feasible. Quasi-experimental designs are a valid option but still not widely used in Portugal. Knowing their use will support the development of this research area. We thus propose a protocol to perform a scoping review aiming at identifying and characterizing the use of quasi-experimental studies to evaluate causal effects of public health interventions in Portugal.

We will include studies assessing causal effects of one or more public health intervention using a quasi-experimental study. PubMed, Scopus, Web of Science and CINHAL will be searched combining free text and controlled vocabulary terms from three concepts: quasi-experimental studies, public health interventions and Portugal. Grey literature will be identified through screening of tables of contents of non-indexed publications and institutional repositories of national Public Health PhD and MSc programmes theses. Title and abstract followed by full-text, double-screening, will be performed.

Searches will be supplemented by reference mining and contact of authors of eligible studies. We will extract information on intervention assessed, study design, statistical analysis approach and reporting guidelines followed using a standardised extraction form.

Results will be synthesized narratively, with descriptive information on areas and characteristics of assessed public health interventions, study design and statistical analysis employed. Whenever possible, descriptions will be categorized and absolute and relative frequencies will be reported.

Our review will identify gaps in the use of quasi-experimental studies thus informing further development of this research area in Portugal.

Acknowledgements

This work have received no funding.

P17

Investigating the association between ambient particulate matter exposure and biomarkers of cardiovascular risk

Vânia Gaio[1,2], Rita Roquette[1,3], Carlos Matias Dias[1,2], Baltazar Nunes[1,2]

[1]Department of Epidemiology, Instituto Nacional de Saúde Doutor Ricardo Jorge IP (INSA, IP), Lisboa, Portugal; [2]NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisboa, Portugal; [3]Nova IMS Information Management School, Universidade NOVA de Lisboa, Lisboa, Portugal

Background and objectives: Ambient particulate matter (PM) is a well-established risk factor to develop cardiovascular diseases but the pathophysiologic mechanisms linking the occurrence of these diseases with PM exposure are still an area of intensive research and scientific debate. This study aims to estimate the effect of PM exposure on some biomarkers of cardiovascular risk, in the adult Portuguese mainland population.
Materials and methods: We used data from 2390 participants of the 1st Portuguese Health Examination Survey (INSEF, 2015) with available information on at least one biomarker of cardiovascular risk and living within a 30-km radius of at least one air quality monitoring station with available PM_{10} measurements. Generalised linear models were used to assess the effect of PM_{10} exposure on biomarkers of cardiovascular risk.
Results: Our results supports the existence of a positive association between PM_{10} exposure and some biomarkers of cardiovascular risk, namely those related to dyslipidaemia (Triglycerides, in people with abdominal obesity: 1.84%, 95%CI: 0.02-3.69, increase per 1µg/m3 PM_{10} increment) and inflammatory response (White blood cells in females: 2.76%, 95%CI: 0.65-4.87, increase per 10 µg/m3 PM_{10} increment and Red cell distribution width in males: 2.96%, 95%CI: 0.80-5.12, increase per 10 µg/m3 PM_{10} increment).
Discussion and conclusions: Exposure to ambient air pollution is largely beyond the control of persons and requires action by public authorities at the national and international levels. Results obtained within this study provide more scientific arguments for taking actions to improve air quality, even when the standard air quality limits are target.

Acknowledgements
The authors are grateful to all the professionals and participants involved in the INSEF. The authors are also grateful to the Portuguese Environmental Agency (APA) for making the air quality data available. INSEF was developed as part of the Pre-defined project financed under the Public Health Initiatives Program. “Improvement of epidemiological health information to support public health decision and management in Portugal. Towards reduced inequalities, improved health and bilateral cooperation” with a 1.500.000€ Grant from Iceland, Liechtenstein and Norway from EEA Grants and the Portuguese Government. The present study was also funded by the Portuguese Foundation for Science and Technology (FCT) (PhD Scholarship Reference: SFRH/BD/129426/2017).

P18

Factors associated with tobacco consumption during pregnancy in Portugal – a cross-sectional study

Filipa Gomes[1], Andreia Leite[2,3,4], Ausenda Machado[2,3,4]

[1]Public Health Unit Professor J. Pereira Miguel, Primary Health Care Cluster Loures-Odivelas, Loures, Portugal; [2]Department of Epidemiology, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal; [3]NOVA National School of Public Health, Public Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal; [4]Comprehensive Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal

Background and objective(s): Tobacco consumption during pregnancy may result in health consequences to both mother and fetus. In Portugal, the dimension of this problem and its factors are still unknow. The aim of this study was to estimate the prevalence of tobacco consumption during pregnancy, in Portugal between 2010 and 2019, and to determine sociodemographic, economic and healthcare access associated factors.
Materials and methods: A cross-sectional study was performed, using 2019 National Health Survey data. Women reporting to have been pregnant between 2010 and 2019 were included. The outcome was tobacco daily or occasional consumption during pregnancy. The exposure variables were age group, nationality, marital status, region of residence, educational level, occupation/professional area, social support, net monthly income, and pregnancy trimester in the first surveillance appointment. Populational prevalence of tobacco consumption and crude and adjusted prevalence ratios with corresponding 95% confidence intervals (95%CI) were estimated, using a Poisson regression model.
Results: Populational prevalence of tobacco consumption during pregnancy was 8.1% (95%CI: 5.6-11.4) (n=744). Prevalence ratio (PR) was higher in young (PR: 1.4, 95%CI: 0.7-2.8), Portuguese (PR: 4.2, 95%CI: 1.1-16.4), single (PR: 1.6, 95%CI: 0.7-4.0) pregnant women, living in Azores (PR: 2.9, 95%CI: 1.0-7.9), with 9th grade (PR: 4.9, 95%CI: 1.8-13.9), manual job (PR: 4.2, 95%CI: 1.2-14.2), weak social support (PR: 3.3, 95%CI: 1.3-8.7), 3rd income quintile (PR: 4.5, 95%CI: 1.0-19.9), 2nd or 3rd trimester appointment (PR: 2.1, 95%CI: 0.8-6.0).
Discussion and conclusion(s): Tobacco consumption during pregnancy is a problem of high magnitude in Portugal, with higher prevalence in more socio-economic disadvantaged pregnant women. Strategies aiming this group should be prioritized.

Acknowledgements
This research received no funding.

P19

INSaFLU-TELEVIR: an open web-based bioinformatics suite for metagenomic virus detection and routine genomic surveillance

João Santos[1], Miguel Pinheiro[2], Daniel Sobral[1], Joana Isidro[1], Carlijn Bogaardt[3,4], Miguel Pinto[1], Ricardo J. Pais[1], Joaquin M. Prada[4], Daniel L. Horton[3], João Paulo Gomes[1] and Vítor Borges[1]

[1]Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [2]Institute of Biomedicine-iBiMED, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal; [3]University of Surrey, Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Surrey, The United Kingdom; [4]University of Surrey, Department of Veterinary Epidemiology and Public Health, School of Veterinary Medicine, Surrey, The United Kingdom

Virus genome sequencing is now a frontline tool for outbreak detection and tracking, being crucial to rapidly characterize circulating viruses and understand their evolutionary trajectories and phenotypic characteristics with relevance for guiding diagnostics, prophylaxis and research. Nonetheless, the implementation of metagenomic virus diagnostics and routine genomic surveillance can be particularly challenging due to the lack of bioinformatics infrastructures and/or expertise to process and interpret next-generation sequencing (NGS) data. In order to begin facing this challenge, we have previously developed INSaFLU (https://insaflu.insa.pt/), which is an open and user-oriented web-based bioinformatics platform that has been crucial for genomics surveillance of influenza, SARS-CoV-2 and, more recently, monkeypox in Portugal. On behalf of the One Health European Joint Programme (OHEJP) TELEVIR (https://onehealthejp.eu/jrp-tele-vir/) project, novel features are being implemented into the "INSaFLU-TELEVIR" bioinformatics toolkit, including a module for virus identification. After reviewing the current state-of-the-art of the field of bioinformatic pipelines for metagenomic virus diagnostics, a start-to-end (from reads quality control to virus detection) modular pipeline has been developed, emphasising accessible end-user reports to promote a robust decision-making on the part of users. A main challenge is the multitude of methods available, so we benchmarked several approaches. We will present preliminary findings and the status of the project. In summary, it is expected that the upgraded INSaFLU-TELEVIR toolkit will supply public health laboratories and researchers with an open and user-friendly framework to potentiate a timely prevention and control of known and emerging viral threats.

Acknowledgements
This study was partially supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme. We also thank the Infraestrutura Nacional de Computação Distribuída (INCD) for providing computational resources for INSaFLU-TELEVIR testing. INCD was funded by FCT and FEDER under project 22153-01/SAICT/2016. The funders had no role in study design, data collection and analysis, decision to publish or preparation of this publication.

P20

WGS-based Dual Strategy Identifies Lipoproteins with PBP Activity as Potential Targets to Enhance Beta-lactam Activity in *Mycobacterium tuberculosis*

Francisco Olivença[1], Alexandra Nunes[2], Rita Macedo[3], David Pires[1,4], Cátia Silveiro[1], Cláudia Ferreira[1], Maria Miragaia[5], Madalena Pimentel[1], Elsa Anes[1], João Paulo Gomes[2], Maria João Catalão[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [2]Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal; [3]National Reference Laboratory for Mycobacteria, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal; [4]Universidade Católica Portuguesa, Católica Medical School, Centre for Interdisciplinary Research in Health, Portugal; [5]Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Oeiras, Portugal

Background and objective: One-third of all antimicrobial resistance fatalities are attributed to tuberculosis (TB). Beta-lactams are the most widely used antibiotics, but their application in TB is limited by inherent characteristics of *Mycobacterium tuberculosis* (*Mtb*). However, the need for a wider therapeutic arsenal has led WHO to consider further research on the role of this class in multidrug-resistant TB regimens as essential. This work aims to identify prospective novel targets that may enhance the activity of beta-lactams in TB.

Materials and methods: A large-scale beta-lactam screening was completed with a collection of *Mtb* clinical strains. Simultaneously, selective pressure was applied to generate beta-lactam-resistant isolates from the reference strain H37Rv. Whole-genome sequencing of all isolates was performed. These outputs were correlated to determine the most relevant hits.

Results: Specific anti-TB drug-resistant strains from the Latin American and Mediterranean sublineage presented significantly lower beta-lactam MICs. Mutations in genes encoding major penicillin-binding proteins (PBPs) and L,D-transpeptidases were uncommon, with increased susceptibility linked to variants in other genes, namely *lpqK*, which encodes a lipoprotein with similarity to PBPs. All mutants yielded by selective pressure shared substitutions in the transcriptional regulator PhoP, and meropenem-resistant mutants possessed an additional mutation in Rv2864c, another lipoprotein with PBP activity. Importantly, clinical strains carrying a substitution within the transpeptidase motif of Rv2864c were significantly associated with higher betalactam susceptibility.

Conclusions: A high conservancy of canonical target genes was observed, reinforcing the potential of beta-lactams against *Mtb*. We show that underexplored players may participate in increased resistance, with lipoproteins with PBP activity emerging as potentially promising therapeutic targets.

Acknowledgements

FO (SFRH/BD/136853/2018) and CS (2021.05446.BD) are recipients of PhD fellowships from FCT. This work was supported by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Switzerland, through Research Grant 2018, and by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through research project PTDC/BIA-MIC/31233/2017, awarded to MJC.

P21

An omics-based look at the role of red blood cells in the vaccine immunization process - COVID-19 vaccines

Joana Saraiva[1], Cristina Valentim-Coelho[1,2], Fátima Vaz[1,2], Marília Antunes[3], Sofia Neves[1,2], Ricardo Peliano[1], Odília Andrade[4], Armandina Miranda[5], Astrid M. Vicente[5], Aryse Melo[6], Carla Roque[6], Raquel Guiomar[6], Lorenza Vantaggiato[1,7], Hugo Osório[8], Mohammed H. Semreen[9,10], Nelson C. Soares[9,10], Deborah Penque[1,2]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; [2]Toxomics-Centre for Toxicogenomics and Human Health, Nova-School, Lisboa, Portugal; [3]Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Portugal; [4]Sector de Apoio Laboratorial, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [5]Departamento de Promoção da Saúde e Prevenção de Doenças, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [6]Departamento Doenças Infeciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [7]Functional Proteomics Lab, Department of Life Sciences, University of Siena, Siena, Italy; [8]Innovation and Health Research Institute (I3S), Porto; [9]Pharmacy-Department of Medicine Chemistry, University of Sharjah, United Arab Emirates; [10]Sharjah Institute for Medical Research, University of Sharjah, United Arab Emirates.

The role of red blood cells (RBC) in the immune system is recognized. Nevertheless, the RBC immunomodulatory role in vaccine immunogenicity is still elusive [1,2]. Taking as a model the COVID-19 vaccines, we aim to investigate whether vaccines induce proteome and/or metabolome changes in RBC able to affect T-cell immune activity, as a mechanistic test for vaccine immunization regulated by RBC. Our ultimate goal is to identify RBC immunomodulators as potential co-adjuvants in the formulation of next-generation vaccines.

A biobank of blood samples collected longitudinally under 'omics' quality control from subjects that underwent vaccination for COVID-19 was generated. This biobank is associated with clinical data, including demographic data, COVID-19 PCR diagnosis, hematological and vaccine immunogenicity data.

Linear Mixed Models were used to analyze the association between biometrical characteristics, health related habits, hematological data, vaccine technology and immunogenicity, along the time-points (t0-t4) under study i.e., before and after (24-72h or 30 days) the vaccine's first/second dose. Statistical analyses were performed using R software version 4.1.2.. Results showed significant differences ($p<0.05$) in a set of hematological variables, including RBC-associated parameters, such as hemoglobin, RDW and MCV, along those different time-points (t0-t4). For some of the hematological parameters, these differences were associated with characteristics such as sex, age, BMI or smoking habits. Preliminary data from proteomics and metabolomics analysis of RBC along the immunization process (t0-t4) will be presented.

This project can bring about evidence-based recommendations intended to optimize vaccine immunization, by recognizing the impact of RBC in the immune system regulation.

References: [1] Morera & MacKenzie. Vet. Res. 2011, 42-89; [2] Antunes et al. Cell Biol. 2011, 89, 111–121.

Acknowledgment: To all volunteers that participate in this study, and to all colleagues from INSA that in somehow contributed to this project. Prof F Leal and Prof S Andrade, Institute Butantan, Brazil and Prof P Picotti, ETH-Zurich are scientific consultants of this project. This Project is supported by INSARJ and Toxomics-FCT grants and has the approval of the Ethical Committee of INSA.I.P.-Lisboa and Centro Nacional e Proteção de Dados, Portugal.

P22

Prophylatic potential of quaternary ammonium surfactants against Streptococcus agalactiae vertical transmission

Carolina Ferreira[1,2], Rita Calado[1,3], João C. Rodrigues[1], Winchill Vaz[3], João P. Gomes[1,4], Otília Vieira[3], Alexandra Nunes[1,4,5]

[1]National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal; [2]NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; [3]NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal; [4]Faculty of Veterinary Medicine, Lusófona University, Lisboa, Portugal; [5]EPCV-School of Psychology and Life Sciences, Lusófona University, Lisboa, Portugal

Streptococcus agalactiae is a leading cause of morbidity and mortality in neonates. Vertical transmission from colonized mothers during labor is currently prevented by intrapartum antibiotics prophylaxis. However, some 2nd-line antibiotics have already been discontinued due to the dramatic increase of antimicrobial resistance, and resistance to 1st-line and even 3rd-line antibiotics has been emerging and increasing. With no vaccine available, it's important to find new alternatives. We have previously shown that the quaternary ammonium surfactant dodecyl-pyridinium bromide (C12PB) is bactericide against S. agalactiae at concentrations that do not affect the commensal flora and epithelial cells of the vaginal mucosa. Herein, we continued C12PB characterization in order to validate it as a good prophylactic compound for the prevention of S. agalactiae vertical transmitted infections.

Our results showed that C12PB has a very low or, at least, slow potential to induce antimicrobial resistance when compared with a commercially available antibiotic. Moreover, no cross-resistance effect that enhance antibiotic resistance was seen for streptococcal populations after prolonged exposure to C12PB. In addition, C12PB

induced very low cytoxicity and/or inflammation when used at concentrations below the surfactant critical micelle concentration and corresponding to the MIC range of several streptococcal isolates.

Overall, these findings highlight C12PB as a promising candidate for the prevention of S. agalactiae vertical transmitted infections, contributing to minimize the global burden of antimicrobial resistance.

Acknowledgements

This work was funded by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through research project PTDC-BIA-MIC-31561-2017, awarded to ANunes. RCalado is a established researcher supported by FCT Project PTDC-BIA-MIC-31561-2017.

P23

Nontuberculous Mycobacteria diversity in Portugal: overview from the last 8 years

S. Carneiro[1,2], A. Santos[1], S. Silva[1], I. Rodrigues[1], A. Silva[3], J.P. Gomes[4], R. Macedo[1]

[1]National Reference Laboratory for Mycobacteria, National Institute of Health Dr. Ricardo Jorge - Lisbon (Portugal); [2]NOVA School of Science and Technology, NOVA University Lisbon - Lisbon (Portugal); [3]National Reference Laboratory for Mycobacteria, National Institute of Health Dr. Ricardo Jorge - Porto (Portugal); [4]Bioinformatics Unit, National Institute of Health Dr. Ricardo Jorge - Lisbon (Portugal)

Background and objective(s): Nontuberculous mycobacteria (NTM) are opportunistic agents, ubiquitous in the environment. True understanding of its epidemiology is not yet established and its clinical impact not fully understood. We aim to characterize the circulation trends of NTM species in Portugal in order to contribute to a better understanding of NTM infections.

Materials and methods: We conducted a national retrospective study using a country representative NTM collection of strains isolated between 2014 and 2020 that is centralized at the National Reference Tuberculosis Laboratory (NRL-TB) of INSA. Positive cultures were identified using GenoType Mycobacterium CM/AS® (Hain Lifescience) and/or Hsp65 DNA sequencing. Social demographic data from patients was analysed and, according to laboratory data solely (number/ body site of sample), the patients were classified into 3 categories: “definite NTM disease”, “NTM colonization” and, “possible NTM disease”.

Results: We were able to identify 910 positive cultures for NTM and 24 different species. Mycobacterium avium complex (MAC), Mycobacterium abscessus-chelonae complex (MABC) and Mycobacterium fortuitum were the species responsible for most of the infections. Geographic distribution of the cases varied and was independent of population density. Lisbon Metropolitan area was the region where most cases (31,0%) occurred followed by the North (25,3%) and Centre (24,4%) regions. North was the region with the highest number of “definite NTM disease” cases.

Conclusions: This work allowed a better knowledge on the circulation of NTM in Portugal. We highlight the need for a systematic approach to diagnose NTM disease and guidelines for uniform reporting in order to assess the real epidemiology of NTM disease.

P24

Multilocus genotyping of Aspergillus fumigatus isolated from patients diagnosed with COVID Associated Pulmonary Aspergillosis (CAPA)

Susana Morais[1,2], Cristina Toscano[3], Helena Simões[2], Dina Carpinteiro[4], Carla Viegas[5,6,7], Cristina Veríssimo[2] and Raquel Sabino[2,8]

[1]Faculdade de Ciências, Universidade de Lisboa; [2] Reference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases, National Institute of Health, Av. Padre Cruz, 1649-016 Lisbon, Portugal; [3] Microbiology Laboratory, Centro Hospitalar Lisboa Ocidental, Hospital Egas Moniz, Portugal; [4] Technology and Innovation Unit, Department of Human Genetics, National Institute of Health, Lisbon, Portugal; [5] H&TRC- Health & Technology Research Center (ESTeSL- Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa), Lisboa, Portugal; [6] NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisboa, Portugal; [7] Comprehensive Health Research Center (CHRC), Lisboa, Portugal; [8] Instituto de Saúde Ambiental, Faculdade de Medicina, Universidade de Lisboa 1649-028 Lisboa, Portugal

Aspergillus fumigatus is an environmental fungus responsible for causing severe invasive infections, especially in immunocompromised individuals. In 2020, during SARS-CoV-2 pandemics, a high number of cases of pulmonary aspergillosis was detected in COVID-19 patients, leading to the definition of CAPA (COVID-19 associated pulmonary aspergillosis). Patients with CAPA harbour many baseline prognostic factors with negative effects on survival, which might be further compromised by azole-resistant Aspergillus isolates.

The aim of this study was to understand the genetic diversity of A. fumigatus isolates collected from CAPA patients in order to perceive the impact of those genotypes in the disease.

With that purpose, we applied a multilocus genotyping methodology (STRAf assay), using tree microsatellite markers (99% discriminatory power) to 100 A.fumigatus isolates. The genetic diversity and the antifungal susceptibility patterns of other clinical and environmental isolates were also characterized.

From the analysed isolates, 85 multilocus genotypes were found. A high diversity of genotypes in CAPA isolates was observed. However, some of those isolates shared genetic similarity since they were collected from patients in the same hospital. Given the ubiquity of A.fumigatus, it is likely that patients may have shared the same contamination source within the hospital environment. In other isolates several multilocus genotypes were shared, reinforcing studies in the One Health Context. In conclusion, CAPA is yet poorly understood due to the lack of studies including isolates collected from COVID patients. Thus, molecular analysis of genetic and epidemiological relationship between those isolates may allow us to assess their potential origin and transmission routes.

Acknowledgements

Professor Lélia Chambel and Professor Pedro Silva from Faculdade de Ciências da Universidade de Lisboa, and Doctor Jaques Meiss from Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital; Centre of Expertise in Mycology Radboudumc/CWZ Nijmegen, The Netherlands.

P25

Update on molecular diversity of Human T Cell Leukemia Virus type 1 circulating in infected individuals diagnosed during the last decade in Portugal

Miriam Quina and Elizabeth Pádua

Reference Laboratory of HIV and Hepatitis B and C, Infectious Diseases Department, National Institute of Health, Lisbon

HTLV-1 is an oncoretrovirus that has been identified as the causative agent -cell Leukemia and chronic neurological diseases (ATLL and HAM/TSP). This retrovirus exhibits a remarkable genetic stability, however, it's classied in several subtypes and subgroups. HTLV-1 infects at leasts 10 million people worldwide, and endemic areas have been recognized. Socio-economic and historical links with countries where this infection is endemic, contribute to the increase in heterogeneity of the resident population in Portugal.

The aim of this study was to update the diversity of HTLV-1 subtypes. Through molecular characterization of viral isolates from 20 individuals diagnosed between 2010 and 2021, LTR and env regions were amplificated to obtain sequences that were analyzed to generate phylogenetic trees.

Only 15% (n=3) individuals were born in Portugal, the others were immigrants (85%; n=17) mostly African (75%; n=15). All strains were classified as Cosmopolitan subtype A. Each half of the individuals' carried either variants of subgroups A or D. In subgroup D, phylogenetic trees showed a segregated cluster with sequences solely derived from individuals infected in Guinea-Bissau (Clade West Africa) that were separated from the north African cluster reference sequences. Despite this study showing higher frequency of isolates classified in this subgroup, the diversity of HTLV-1a variants shows no increase since the last molecular study in 2009. However, risk behavior or silent dissemination with a broad diversity of viruses, may occur throughout the time due to continuous increase of population heterogeneity. Therefore, periodic surveillance of HTLV-1 infection remains necessary.

P26

Exploring the diversity of microbiome in aquaculture

Daniel Olivença[1], Mónica Azevedo[2], Vanessa Salgueiro[2,3,4], Eberhard Voit[1], Vera Manageiro[2,3,4], Manuela Caniça[2,3,4]

[1]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University - Atlanta, Georgia (United States); [2]National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR/HAI), Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge (INSA), Lisbon, Portugal; [3]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Porto, Porto, Portugal; [4]AL4AnimalS, Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal

Background and objectives: Aquaculture is growing more rapidly than all other animal food-production sectors. The intensive use of antibiotics to prevent infectious diseases, causing significant stock losses and problems with aquatic-farmed animal welfare, might constitutes a potential human health hazard that has received quite little attention when compared to terrestrial-farm animals. The aim of this work was to determine the microbial biodiversity and the antibiotic resistance genes (ARG) that are promoted in different aquaculture environments, and to establish the linkage between ARG and the comparison between animals and environment.
Materials and methods: Four fresh samples representative of the main aquaculture-producing animals in Portugal (saltwater cultured seabream and mussels) and respective farming water from one culture were collected and studied by amplicon sequencing and NGS target enrichment. The 16S and ARG present were studied for all samples. Specific tools were used for bioinformatics and statistical analysis.
Results: The study showed that samples of water are the most diverse in terms of microbial species, followed by mussels. We identified important ARG and genes coding to virulence factors, biofilms, antiseptic-, dye- and heavy metal resistance, as well as mobile genetic elements.
Conclusions: Data suggests a separation between the water environment and organisms cultivated in aquaculture, both concerning the microbiome and resistome. This study also shows the antibiotic resistance associated with different resistance mechanisms and other relevant genes, which can promote co-selection; these genes are in different aquaculture settings, thereby allowing for antibiotic resistance to develop and spread via food-fish and the environment, which might result in significant human health threats.

Funding: V.S. has her Ph.D. fellowship granted by FCT (Fundação para a Ciência e a Tecnologia) [SFRH/BD/133100/2017] co-financed by European Social Fund and the Operational Program for Human Capital (POCH), Portugal. The work was supported with funding from FCT/MCTES [UIDB/00211/2020] through national funds.

P27

Isolation and characterization of Campylobacter spp. from surface and recreational Waters

Mónica Azevedo[1], Filipa Vale[2], Mónica Oleastro[3]

[1]Faculty of Medicine, University of Lisbon, Lisbon, Portugal; [2]Pathogen Genome Bioinformatics and Computational Biology Unit, Research Institute For Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon Portugal; [3]National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal

Background and objectives: Campylobacteriosis is the most common cause of acute bacterial gastroenteritis worldwide. Environmental waterbodies have been implicated in the transmission of Campylobacter, also being considered reservoirs of resistome. Through this study we characterized C. jejuni and C. coli from surface and recreational waters of Lisboa and Vale do Tejo.

Materials and methods: Forty-nine samples were collected from 40 sampling sites and subjected to selective culture with enrichment for Campylobacter spp. Antibiotic resistance profiles were assessed by agar diffusion testing and genomic analysis was performed using different tools (as PubMLST, chewBBACA, PHYLOViZ, AMRFinderPlus, and RAST).

Results: Our results show a prevalence of 29% (14/49) of Campylobacter spp., and a significant association with lakes, creeks, and rivers. Fourteen C. jejuni were isolated from four samples and 21 C. coli from six samples. These were mostly susceptible, though 20% were resistant to ampicillin and 17% to tetracycline. In silico analysis allowed identification of antibiotic resistance genes (ARGs) (as tet(O) and genes encoding β-lactamases) and of virulence genes and structures (including cadF, jlpA, porA, ciaB, flaC, cdtABC, and functional type IV and type VI secretion systems).

Discussion and conclusions: The surface and recreational waters integrated in our study didn't contribute to contemporary campylobacteriosis cases. Still, these water isolates were previously identified in clinical cases and outbreaks of Campylobacter, also harbouring ARGs and virulence factors, emphasizing the pathogenicity of water isolates. Further studies with bigger sample sizes and considering different settings, as agriculture and livestock related waterbodies, are important for more robust conclusions.

Acknowledgements and Funding

The project was performed under supervision of Dr. Filipa F. Vale and Dr. Mónica Oleastro and was financially supported by Instituto Nacional de Saúde Dr. Ricardo Jorge and co-financed by One Health European Joint Programme project, Grant Agreement no. 773830.

P28

Detection and genotyping of hepatitis B virus in samples from vulnerable population with risk behavior to acquire viral infectious diseases

Carolina Silva and Elizabeth Pádua

Reference Laboratory of HIV and Hepatitis B and C, Infectious Diseases Department, National Institute of Health, Lisbon

Vaccination against hepatitis B virus (HBV) was implemented in the Portuguese National Vaccination Plan in 1994 for adolescents, and in 2000 for newborns. Still, vulnerable populations, namely injecting drug users (IDUs) and inmates, are at risk for acquiring viral infections due to their harmful behaviors. This study aimed to detect HBV DNA in IDUs with Hepatitis C antibodies, identify HBV genotypes circulating in inmates, and assess treatment-resistance mutations. Samples from two subgroups of IDUs, distributed according to geographic origin, 140 born in Portugal (before 1971) and 31 born outside Portugal, and from 41 inmates chronically infected with hepatitis B were analyzed.
Methodologies included screening of HBsAg in plasma pools, DNA extraction from positive plasmas, PCR amplification, and sequencing of S and Core regions of HBV genome. Sequences were genotyped by phylogenetic analysis and checked for treatment-resistant mutations. In Portuguese IDUs (average age of 50 years), who were probably not vaccinated, HBV DNA was detected in 2 (1.4%) individuals, one of them infected with genotype E. In samples from IDUs born outside Portugal (45.2% were Africans), no HBV infection was detected. Inmates showed a higher diversity of genotypes (A, 41.5%; D, 29.3%; E, 26.8%; F, 2.4%). These results included Portuguese inmates (48.8%; average age 37.4 years), who were expected to be vaccinated in adolescence. No treatment-resistant mutations were detected.
HBV surveillance combined with strategies to vaccinate vulnerable populations is essential to prevent the spread of the virus among these populations, who do not easily adhere to conventional health structures.

Acknowledgements
Rodrigo Coutinho, on behalf of Ares do Pinhal Association for Social Inclusion, Lisbon Luís Vieira, on behalf of the Technology and Innovation Unit of the National Institute of Health, Lisbon.

P29

Survey of genomic features that putatively contribute to antimicrobial resistance in Chlamydia trachomatis

Jorge Costa da Silva[1], Zohra Lodhia[1], Dora Cordeiro[1], Suza Almeida[1], Inês João[1], Cristina Correia[1], Luís Vieira[2], João Paulo Gomes[3], Vítor Borges[3], Maria José Borrego[1]

[1]National Reference Laboratory for Curable Sexually Transmitted Infections, Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal; [2]Innovation and Technology Unit, Department of Human Genetics, National Institute of Health, Lisbon, Portugal; [3]Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal

Chlamydia trachomatis (CT) is the leading cause of bacterial sexually transmitted infections, with an estimation of 131 million new episodes per year. CT can be classified into 15 main genotypes (A-C, D-K, L1-L3), based on polymorphisms of the ompA gene. These ompA-genotypes correlate with specific tissue tropisms for different mucosal sites. Chlamydial infections can be treated with antibiotics such as macrolides (azithromycin), tetracyclines (doxycycline) or fluoroquinolones. However, CT can be detected post-treatment, which is often related with reinfection or lack of treatment compliance. Since antimicrobial resistance (AMR) cannot be excluded, it is a phenomenon that requires further elucidation. As routine diagnosis of CT does not entail culture methods, which impedes phenotypic assays for appraising the occurrence of antimicrobial resistance, the need for a system that provides such information is pivotal. A novel Multiplex PCR approach was developed for amplification of target genes potentially driving AMR (23S rRNA, gyrA and parC) and of genes that allow inferences on the genotype and genomic backbone of CT (CT105, CT442 and pmpH), followed by NGS and bioinformatic analysis. A total of 576 strains were studied. Mutation survey revealed no mutants resistant to macrolides, but 4 mutants were identified which could be resistant to fluoroquinolones. Amplification of CT105, CT442 and pmpH loci revealed that these target loci, together with ompA, could constitute a more robust typing system of CT strains, particularly important to discriminate L1-L3 (LGV) strains.

P30

NiPharmins as efficacious prophylactic options to prevent Staphylococcal Infections

Bárbara Alves[1,2], Rita Calado[1], João C. Rodrigues[1], João Jorge[3], Sházia Abdul[3], Íris Guerreiro[3], Raquel Portela[2,4], Rita Sobral[2,4], Sofia Ramos[3], João P. Gomes[1,5], Nuno R. Candeias[6], Alexandra Nunes[1,3,5]

[1]National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal; [2]NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; [3]EPCV-School of Psychology and Life Sciences, Lusófona University, Lisboa, Portugal; [4]Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; [5]Faculty of Veterinary Medicine, Lusófona University, Lisboa, Portugal; [6]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal

The rapid increase of Staphylococcus aureus antimicrobial resistance together with their impact in healthcare, highlights the urgent need of alternative therapeutic compounds, eventually with new modes of action. We have recently demonstrated that novel alkylaminophenols (NiPharmins) are promising effective antibacterial agents against several multi-resistant S. aureus, including MRSA, at concentrations that are not cytotoxic. Herein, we intended to contribute for NiPharmins validation as efficacious prophylactic options against staphylococcal infections.

By using an in vitro selective pressure approach, S. aureus isogenic clones continuously propagated without and under a NiPharmin sub-letal concentration showed unchanged MIC after 50 passages, pointing to a lack (or very low) potential to induce antimicrobial resistance when compared to the conventionally used rifampicin, for which resitance was seen soon after the 2nd passage, reaching MICs up to 4000-fold higher for some clones at the end of the assay. Moreover, antimicrobial susceptibility testing of the final S. aureus populations against a panel of 16 antibiotics revealed that their continuous exposure to a sub-letal concentration of NiPharmin did not promote any cross-resistance phenomenon (enhanced resistance) to conventional antibiotics when compared to the initial bacterial clone, which validates its prolonged used. Furthermore, NiPharmins presented an antibacterial efficacy of 75% soon after 3h of compounds' exposure, being maximum at 6h, allowing to narrow down the clinical situations in which these compounds may be applied.

Overall, these findings were undoubtedly a step forward in NiPharmin's validation as promising therapeutic alternative for the prevention of S. aureus infections, opening avenues for future studies.

Acknowledgements

This work was funded by Jane and Aatos Erkko Foundation (Decision 287954).

P31

Genomic epidemiology of SARS-CoV-2 in Portugal: supporting evidence-informed public health decision-making

Vítor Borges[1], Joana Isidro[1], Rita Ferreira[1], Sílvia Duarte[2], Daniela Santos[2], Luís Vieira[2], Miguel Pinto[1], Luís Coelho[1], Verónica Mixão[1], Raquel Neves[1], Alexandra Nunes[1], Daniel Sobral[1], Raquel Guiomar[3], Portuguese network for SARS-CoV-2 genomics (Consortium), João Paulo Gomes[1]

[1]Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [2]Innovation and Technology Unit, Department of Human Genetics; National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal; [3]National Reference Laboratory for Influenza and other Respiratory Viruses, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal.

The first cases of COVID-19 in Portugal were reported at the beginning of March 2020, with a following exponential increase. As the National Reference Laboratory, INSA established a nationwide network involving >100 laboratories in the early pandemics, which provides 300-600 positive samples weekly, culminating in an ongoing genomic surveillance strategy for SARS-CoV-2.

Sample processing consists in cDNA generation through RT-PCR, followed by amplicon-based whole-genome amplification and sequencing. Data are analyzed using the INSaFLU pipeline (https://insaflu.insa.pt/), from quality control to mutation detection, consensus generation/curation, "Pango" lineage classification, among other features/outputs. Integrative phylogenetic and geotemporal data analysis is performed using Nextstrain tools (https://nextstrain.org/).

We present the implemented strategy for genomic epidemiology of SARS-CoV-2 in Portugal, demonstrating its impact on outbreak monitoring and control. Among its several outcomes, we highlight the continuous generation of data (weekly reported at https://insaflu.insa.pt/covid19/) towards an evidence-based decision-making, triggering public health and social measures with direct impact on epidemic evolution. It allowed monitoring the emergence and dissemination of variants of interest/concern, contributing to assess their impact on vaccine effectiveness and disease severity. This work also contributed to several parallel studies supporting specific clinical and public health investigations.

To date, ~44000 genomes have been publicly released by INSA, showing an unprecedented effort in the infectious diseases field in Portugal, and highlighting the need for systematic and geographically-representative surveillance to aid public health efforts for outbreak tracking and control. It shows how genomic data can strongly support/complement epidemiological investigation and public health actions, guiding preparedness actions to face future epidemics.

Acknowledgements

The early stage of this study was co-funded by Fundação para a Ciência e a Tecnologia and Agência de Investigação Clínica e Inovação Biomédica [grant number 234_596874175] on behalf of the Research 4 COVID-19 call. Some infrastructural resources used in this study come from the GenomePT project (POCI-01-0145-FEDER-022184). The acquisition of WGS-associated equipment and reagents used in this study (including the Illumina NextSeq 2000) was funded by the HERA project (Grant/2021/PHF/23776) were partially supported by the European Commission through the European Centre for Disease Control and Prevention.

P32

Assessment of the transmission dynamics of *Clostridioides difficile* in a farm environment reveals the presence of a new toxigenic strain connected to swine production

Frederico Alves[1†], Alexandra Nunes[1,2,3†], Rita Castro[1], António Sequeira[4], Olga Moreira[4], Rui Matias[1], João Carlos Rodrigues[1], Leonor Silveira[1], João Paulo Gomes[1,2], Mónica Oleastro[1]

[1]Infectious Diseases Department, National Institute of Health Dr Ricardo Jorge (INSA), Lisbon, Portugal; [2]Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal; [3]CBIOS – Lusófona University Research Centre for Biosciences & Health Technologies, Lisbon, Portugal; [4]National Zootechnical Station, National Institute for Agrarian and Veterinarian Research, Santarém, Portugal; [†] Equal contribution

The increase in community-acquired *Clostridium difficile* infections and its' isolation from different animal and environmental reservoirs reveal the importance of better understanding the intercommunity transmission cycle. This study aimed at establishing a transmission network, using an animal production unit as a proof-of-concept, as well as assessing the zoonotic potential and genomic features of the dominant clones.
Samples were collected from animal, human and environmental compartments. *C. difficile* isolates were characterized for toxigenic profile by multiplex-PCR, while genetic diversity was evaluated by PCR-ribotyping and whole genome-based analysis. 188 samples were collected. The overall *C. difficile* prevalence was 37.2% (70/188), and included samples from environmental (58.3%, 35/60) and animal (31.5%, 35/111) origin. A predominant RT033 clone was found in almost 90% of the positive samples, including samples from all compartments connected to the pig production unit, with SNV-based analysis supporting clonal transmission (mean distance of 0.1±0.1 core-SNVs). The isolates from this clone (PT RT033) were positive for all *C. difficile* toxin genes (*tcdA, tcdB, cdtA/cdtB*) and its' phylogenetic positioning was clearly distinct from the classical RT033. PT RT033 shares genomic features with several RTs from the clade 5 Sequence Type (ST) 11, like the presence of a complete pathogenicity locus more similar to the one found in toxigenic strains than in the less virulent classical RT033 (*tcdA-, tcdB-, cdtA+/cdtB+*).
This study constitutes the first report of a toxigenic RT033 clone, adds to the overall knowledge on Clade 5 sequence type 11, considered the C. difficile evolutionary lineage with the highest zoonotic potential.

Acknowledgements
This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 773830: One Health European Joint Programme. FA and RC were recipients of fellowships from the same programme. WGS was performed at Unit of Innovation and Technology, Human genetic Department, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.

P33

Remote monitoring of disease vector mosquitoes with a new optical sensor system for automatic classification

Manuel Silva, Maria João Alves, Fátima Amaro, Líbia Zé-Zé, Hugo Osório

Centre for Vectors and Infectious Diseases Research/National Institute of Health Doutor Ricardo Jorge, Avenida da Liberdade 5, 2965-575 Águas de Moura, Portugal

Mosquitoes represent a major threat to public health given their ability to transmit several pathogens. Some species of Aedes can transmit viruses such as dengue, Zika, or chikungunya. Aedes aegypti was first identified on Madeira Island in 2005. Seven years after its identification, the first dengue outbreak occurred on this island. Aedes albopictus was first recorded in 2017 and its dissemination is in progress. Prevention of vector-borne diseases largely depends on effective and sustainable vector surveillance.
Mosquitoes both collected in the field and reared in insectary conditions were used to develop classification models for the automatic identification of mosquito species using a novel optical sensor system coupled to a standard mosquito suction trap. Field sensors were also deployed in Madeira and Algarve regions for real pilot trials.
A total of 10621 mosquitoes of seven species were tested.
Two categories were considered: target species, Ae. albopictus, Ae. aegypti, An. gambie; and non-target species, Cs. longiareolata, Cx. theileri, Cx. laticinctus, Cx. hortensis.
Preliminary results of this work indicate the capacity of the sensor distinguishing males from females with a 99% accuracy and distinguishing between the different genus of mosquitoes with more than 90% accuracy.
Preventing and controlling vector borne diseases is urgent. WHO promotes a strategy to strengthen the control over vector-borne diseases worldwide. Implementing a system of real-time surveillance using this technology is a large step in achieving this goal. Moreover, environmental data will be used to study the vector capacity of Ae. albopictus in Portugal to dengue, Zika and chikungunya.

Acknowledgements
This work is under the VECTRACK project (Earth observation service for preventive control of insect disease vectors) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 853758.

P34

Occupational secondhand smoke exposure: a proteomic approach

Sofia Neves[1,2*], Solange Pacheco[1*], Fatima Vaz[1,2], Peter James[3], Tania Simoes[4], Deborah Penque[1,2]

[1]Laboratory of Proteomics, National Institute of Health Dr. Ricardo Jorge, INSA I.P, Lisbon Portugal; [2]ToxOmics- Center of Toxicogenomics and Human Health, Universidade Nova de Lisboa, Portugal; [3]Protein Technology Laboratory, Department of Immunotecnology, Lund University, Sweden; [4]CECAD Cologne-Excellence in Aging Research University of Cologne, Germany; [*]both authors contributed equally to this study

Non-smokers exposed to Second HandSmoke (SHS) are at risk of developing tobacco-smoke diseases. The biological mechanisms underlying the SHS-induced pathogenesis, that would allow risk assessment are still not fully elucidated. To respond to these public health needs, we carried out a proteomic study on a group of restaurant employees occupationally exposed to SHS.

Workers (n=52) from 25 restaurants in Lisbon were recruited for clinical evaluation and nasal epithelium sample collection for proteomics study. The subjects were classified as smokers (S, n=8), never-smokers (N, n=11) or former-smokers (F, n=8), non-exposed, and never-smokers (NE, n=11) or former-smokers (FE, n=10), exposed to SHS. All subjects were healthy showing no significant differences in pulmonary function. Protein extracts from nasal samples were prepared and analyzed by shotgun proteomics and the identified proteins analyzed by bioinformatics. The acquired knowledge was integrated into functional networks.

The results indicated that the NE proteome is mostly associated with the biological terms “Lactate dehydrogenase complex”, “Pentose-phosphatase shunt” and “Glutathione peroxidase activity”. The FE subjects presented a proteome enriched with terms as “L-Lactate dehydrogenase complex” and “Peroxisome”. These data suggested that “Hypoxia” and “Detoxification” are the cellular process mostly modulated in both never or former smokers exposed to SHS.

“Central carbon metabolism in cancer” and “Nucleossome histone variants” were exclusively identified in FE subject’s proteome, probably associated with harmful cumulative biological processes resulting from previous smoking habits and current exposure to SHS.

These findings are dependent of the small dimension of the analyzed groups and further validation studies are needed.

Acknowledgements

To all restaurants and their workers who voluntarily participated in this study. This work was supported by the Gulbenkian Foundation, the Portuguese Mass Spectrometry Network (RNEM), the Administração Central do Sistema de Saúde de Portugal (SNS) by National Institute of Health Dr. Ricardo Jorge, INSA and the Centre for Toxicogenomics and Human Health - ToxOmics. SN was granted with a PhD FCT fellowship.

P35

Snapshot of polycyclic aromatic hydrocarbons, microplastics and biofilms occurence at touristic spots of Alqueva surface water during 2021

Ana Raposo[a], Catarina Mansilha[b,c], Alexander Veber[d,e], Armindo Melo[b,c], João Rodrigues[f], Rui Matias[f], Helena Rebelo[a], José Grossinho[a], Manuela Cano[a], Cristina Almeida[a], Isabel D. Nogueira[g], Ljiljana Puskar[e], Ulrich Schade[e], Luisa Jordão[a]

[a]Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Saúde Ambiental, Av Padre Cruz, 1649-016 Lisboa, Portugal; [b]Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Saúde Ambiental, Rua Alexandre Herculano 321, 4000-055 Porto, Portugal; [c]Associated Laboratory for Green Chemistry (LAQV) of the Network of Chemistry and Technology (REQUIMTE), University of Porto, Praça Gomes Teixeira, 4051-401 Porto, Portugal; [d]Humboldt Universität zu Berlin, Institute of Chemistry, Brook-Taylor Strasse 2, D-12489 Berlin, Germany; [e]Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Infrared Beamline IRIS, Albert-Einstein-Strasse 15, D-12489 Berlin, Germany; [f]Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Doenças Infeciosas, Av Padre Cruz, 1649-016 Lisboa, Portugal; [g]Instituto Superior Técnico, MicroLab, Av Rovisco Pais, 10049-001 Lisboa, Portugal

The occurrence of Persistent environmental pollutants, such as polyciclic aromatic hydrocarbonets (PAHs) and microplastics (MPs), in freshwater is a major concern. Here we aim at characterizing the occurrence of these pollutants, physicochemical and microbiota of superficial freshwater samples collected at 3 spots of Alqueva.

Physicochemical properties were mainly determined by UV-Visible spectrophotometry and electrometric methods. PAHs were determined by dispersive liquid-liquid microextraction followed by GC-MS. Bacteria and fungi were isolated using selective media followed by identification by biochemical methods. Scaning electron microscopy and infra-red microscopy were used to identify biofilms and MPs, respectively.

Physicochemical analysis of the water revealed small variations in water quality between seasons, and were indicative of a low eutrophication level. Low concentrations of PAHs were detected being higher concentrations and compounds diversity found during Spring. MPs were detected in all samples except one, being low/high density polyethylene the most frequent. Being among the polymers with higher demand in Europe and major contributors for plastic waste, these results were expected and are indicative of an incorrect waste disposal problem.

Plastic polymers were more prone to biofilm colonization than natural materials, and the biofilms detected were more complex, with higher microbial diversity and richer in extracellular polymeric material. The identification of several species of Bacillus, Klebsiella pneumoniae and E.coli, among other species of microorganisms related to plastic degradation in these water samples leads us to speculate that they could colonize the polymers and contribute to their fragmentation and degradation, suggesting an environmentally friendly strategy to boost bioremediation.

Acknowledgements

Infrared spectroscopy measurements were carried out at the IRISbeamline at the BESSY II electron storage ring operated by the Helmholtz-Zentrum Berlin für Materialien und Energie (Proposals No 202-10133-ST/UR, 2020 and 212-10726-ST/UR, 2021). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreementNo 730872. We thank INSA for financial support [2020DSA1756, 2020], Carla Ferreira (INSA, DSA) for technical support during the sampling campaigns, Isabel Prata and João Sousa (INSA, DSA) for technical support during the evaluation of physical and chemical parameters.

P36

Thermal comfort and climatic conditions effects on older adults in naturally ventilated nursing homes

Daniela Almeida[1], Nuria Forcada[2], Filipa Esteves[1,3,4], Paula Neves[1], João Paulo Teixeira[1,3,4], Ana Mendes[1,3,4]

[1]Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal; [2]Department of Project and Construction Engineering (DPCE), Group of Construction Research and Innovation (GRIC), Universitat Politècnica de Catalunya. (UPC), Barcelona, Spain; [3]EPIUnit – Institute of Public Health, University of Porto, Porto, Portugal; [4]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Porto, Portugal

Life expectancy increases but not necessarily the years of a healthy life. The interaction between microclimate and older people's health could impact the worsening of their chronic illness. Two Mediterranean climates (Csa-m, Csa-c) and an oceanic climate (Csb) were analysed through a field study based on environmental measurements and a survey during naturally ventilated seasons. To assess the indoor thermal environment in common spaces, a Delta Ohm HD 32.3 instrument was used. The instrument was located approximately 1.5 m above ground and recordings were taken in each room, according to ASHRAE 55, at the same time as the surveys were administered. For the outdoor dry temperature (Tout) and relative humidity (RHout), data from the weather stations closest to each building were used.
The conclusions drawn from the analyses and discussions of this study are as follows: (i) Elderly from the different climates has different thermal perception; (ii) Apart from the temperature, outdoor humidity also influences indoor thermal comfort; (iii) Neutral temperature for elderly depends on the climate; (iv) High indoor humidity implies higher heat sensation; (v) The real elderly thermal sensitivity was lower than PMV prediction model.

Acknowledgements
The authors thank the International Center on Aging (CENIE), the General Foundation of the University of Salamanca (FGUSAL) and the Programa de Cooperación Interreg V-A España – Portugal (POCTEP) 2014–2020 and the FEDER funds. We would also like to extend our appreciation to all the nursing homes and caregivers, maintenance staff and elderly people who participated in this project.

P37

Biomonitoring Portuguese Wildland Firefighter's in Pre-fire season: Preliminary results

Filipa Esteves[1,2,3], Solange Costa[1,2], Joana Madureira[1,2], Joana Pires[1,2], João Paulo Teixeira[1,2]

[1]Environmental Health Department, National Institute of Health. Rua Alexandre Herculano, 321, 4000-055 Porto, Portugal; [2]Laboratory for Integrative and Traslational Research in Population Health (ITR). Rua das Taipas 135, 4050-600 Porto, Portugal; [3]Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto. Alameda Prof. Hernâni Monteiro, 4200-072 Porto, Portugal

In June 2022, occupational exposure as a firefighter was classified as "carcinogenic to humans" by the International Agency for Research on Cancer.
The general aim of Bio4FOX study is to characterize the exposure (pre- and during wildfire season) and identify a panel of biomarkers for the surveillance of Portuguese wildland firefighters' health.
A total of 170 wildland firefighters (83% male and 17% females; mean age 37.9±10.8) from 14 fire stations located in the northern region of Portugal were enrolled in prefire season (2021).
Data from the Buccal Micronucleus Cytome Assay (BMCyt) suggest that firefighters belonging to Permanent Intervention Teams (full time firefighters) showed an increased frequency of karyolitic cells (cell death biomarker) ($p<0.05$). Regarding lifestyle, it was observed a positive correlation between NBUD frequency (DNA damage) and the number of years as a smoker ($r=0.33$; $p<0.05$). It was also observed a relationship between certain dietary patterns and BMCyt outputs; coffee intake and higher consumption of smoked foods were related with high frequencies of karyolitic and pycnotic cells (cell death biomarker), correspondingly ($p<0.05$). On the other hand, vegetables consumption shown to have a protective role on cells decreasing the frequency of pycnotic cells ($p<005$). In addiction, decreased levels of MN frequency (DNA damage) were found in individuals who reported to take nutritional supplements ($p<0.05$).
This research project is anticipated to lead to: a better characterization of firefighters' occupational exposure; the development of preventive measures and policies among the sector and the identification of future research needs for the wildland fire community.

Acknowledgements
This work received financial support by the project PCIF/SSO/0017/2018 by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Filipa Esteves, recipient of PhD grant UI/BD/150783/2020, is supported by FCT and by the European Social Fund (ESF). Joana Madureira, under the grant SFRH/BPD/115112/2016, is supported by FCT and by ESF, through Programa Operacional Capital Humano (POCH). The authors also acknowledge the ISPUP (UIDB/04750/2020) and ITR (LA/P/0064/2020).

P38

Reducing mercury exposure during pregnancy in Portugal through suitable seafood dietary advice: contribution from the HBM4EU-mom project

Maria Peres[1], Ricardo Assunção[2,3,4], Susana Santiago[1], Carla Luísa Nunes[1], Marta Esteban López[5], Noelia Dominguez Morueco[5], Susana Pedraza Diaz[5], Miguel Juliá[5], Argelia Castaño[5], Þórhallur Ingi Halldórsson[6], Eva Jacobsen[6], Catherine Gabriel[7], Achilleas Karakoltzidis[7], Spyros Karakitsios[7], Despina Giannadaki[8], Adamos Hadjipanayis[9], Elena Anastasi[8], Chrysanthi Thoma[8], Janja Snoj Tratnik[10], Milena Horvat[10], Laura Rodríguez Martín[11], Liese Gilles[11], Eva Govarts[11], Dimosthenis Sarigiannis[7], Andromachi Katsonouri[8], Sónia Namorado[1,2]

[1]National Institute of Health Dr. Ricardo Jorge; [2]Public Health Research Centre & Comprehensive Health Research Center; [3]Centre for Environmental and Marine Studies, University of Aveiro; [4]Instituto Universitário Egas Moniz; [5]National Centre for Environmental Health, Instituto de Salud Carlos III; [6]Faculty of Food Science and Nutrition, University of Iceland; [7]Aristotle University of Thessaloniki & HERACLES Research Center on the Exposome and Health, Center for Interdisciplinary Research and Innovation; [8]Cyprus State General Laboratory; [9]Larnaca General Hospital; [10]Jožef Stefan Institute, Department of Environmental Sciences; [11]Flemish Institute for Technological Research.

Background: Methylmercury contaminated seafood is the main source of mercury exposure in Europe, and exposure is higher in countries with higher seafood consumption, such as Portugal. Pregnant women and their foetuses are particularly vulnerable due to the effects of mercury on the central nervous system in the prenatal stage.
Objectives: To characterize mercury exposure of pregnant women in Portugal and evaluate the impact of a dietary intervention designed to reduce exposure.
Methods: HBM4EU-mom was a pilot randomized intervention study aiming to reduce methylmercury exposure of pregnant women in high seafood-consuming European countries. Women received recommendations on safe seafood intake during pregnancy (intervention) vs. standard care (control). Each study phase included a measurement of total mercury in hair and a questionnaire on health, nutrition and lifestyle. Phase 1 was at ≤20 weeks of pregnancy (n = 135) and phase 2 occurred ≥12 weeks later (n = 113).
Results: Total mercury in hair was higher in the intervention group (GM: 1.61 µg/g in phase 1 vs. 1.39 µg/g in phase 2) compared to the control group (GM: 1.58 µg/g in phase 1 vs. 1.35 µg/g in phase 2). 36% of all samples exceeded EFSA's health-based guidance value (1.8 µg/g) in phase 1 vs. 27% in phase 2.
Conclusions: Seafood intake and mercury exposure were high in Portugal, but mercury levels decreased in phase 2 in both groups. Results show it is crucial to continue to raise awareness to the mercury-related risks of seafood consumption during pregnancy, but also to its nutritional benefits.

Acknowledgements
HBM4EU-mom was co-funded under HBM4EU by the Horizon 2020 programme (grant agreement No 733032) and by the partner institutions of each participating country.