



UNIVERSIDADE  
DE ÉVORA

**III International Meeting of the  
Portuguese Society of Genetics**

27<sup>th</sup> > 28<sup>th</sup> June 2022

# **III International Meeting of the Portuguese Society of Genetics**

**27<sup>th</sup> and 28<sup>th</sup> June 2022**

**Auditorium of the Colégio Espírito Santo, University of Évora**

# **Book of abstracts**

---

(Draft Version)



**Title:** III International Meeting of the Portuguese Society of Genetics

**Editors:**

Ana Alexandre

Fátima Duarte

Hélia Cardoso

**Address:**

Universidade de Évora,

Largo dos Colegiais, 2

7004-516 Évora

**Published:**

UE – Universidade de Évora

Copyright © 2022, all rights reserved

ISBN (to be added)



Dear participants,

It is our great pleasure to welcome you to the **III International Meeting of the Portuguese Society of Genetics**, held in Évora on the 27<sup>th</sup> and 28<sup>th</sup> of June 2022. We have put together a two-day program, organised in five different sessions and focused on encouraging scientific discussion.

As in the previous editions, this Meeting is dedicated those who work or are interested in the different areas of Genetics. The program will reflect the diversity of subjects within Genetics, with special focus on Animal Genetics, Plant Genetics, Microbial Genetics, Biomedical Genetics and Evolutionary Genetics. Invited speakers with outstanding careers will set the tone to a Meeting that we hope will be a great opportunity to present and discuss the latest advances in Genetics.

The III International Meeting of the Portuguese Society of Genetics (IMPSG) will take place at an Auditorium located at the most emblematic building of the University of Évora, the Colégio Espírito Santo.

The organizing committee, on behalf of the University of Évora and of the Portuguese Society of Genetics, welcomes researchers and also students working in the field of Genetics to this two-day Meeting, in which we will get back to the interactions of an in-person meeting, sharing experiences and knowledge, and enjoying the scientific atmosphere of Évora.

Finally, we wish to thank the scientific committee for their key role in many important aspects of this meeting. We hope that all the participants enjoy the IMPSG and appreciate the beautiful city of Évora, an UNESCO World Heritage. In this book of abstracts you will find all the detailed information regarding the meeting, including the scientific program and a list of all participants.

Welcome to Évora!

The Organizing Committee,

Ana Alexandre

Hélia Cardoso

Fátima Duarte



## COMMITTEES

### Organising Committee

Ana Alexandre, MED-CHANGE, Universidade de Évora

Fátima Duarte, MED-CHANGE, CEBAL

Hélia Cardoso, MED-CHANGE, Universidade de Évora

### Scientific Committee

Ana Gabriela Henriques

Grupo de Neurociências e Sinalização, Instituto de Biomedicina, Departamento de Ciências Médicas, Universidade de Aveiro, Portugal

Ana Teresa Maia

CINTESIS-Universidade do Algarve

Ana Usié

Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/ Polytechnic Institute of Beja (IPBeja)  
Group of Animal Genomics and Bioinformatics

Andreia Figueiredo

Assistant Professor & Grapevine Pathogen Systems Lab Head

Biosystems & Integrative Sciences Institute, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa

Diana Lousa

Researcher at Cláudio M. Soares Lab

ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier

Leonor Cancela

Full Professor, Faculty of Medicine and Biomedical Sciences, University of Algarve

Luísa Mesquita Pereira

i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto

Ipatimup – Instituto de Patologia e Imunologia Molecular da Universidade do Porto

Margarida Casal

Centro de Biologia Molecular e Ambiental (CBMA), Instituto de Inovação e Ciência para a Bio-sustentabilidade (IB-S),

Departamento de Biologia, Universidade do Minho, Braga

Patrícia Beldade

Assistant Professor at the Faculty of Sciences of the University of Lisbon

Principal Investigator at the Centre for Ecology, Evolution, and Environmental Changes

Paulo De Oliveira

Biology Department, University of Évora

Raquel Chaves

CAG – Laboratory of Cytogenomics and Animal Genomics | Group Leader

DGB – Department of Genetics and Biotechnology

Associate Professor with Habilitation, Universidade de Trás-os-Montes e Alto Douro

PI/GER – Gene Expression and Regulation Group do BioISI – Biosystems & Integrative Sciences Institute,  
Faculdade de Ciências da Universidade de Lisboa

Sandra Correia

Center for Functional Ecology, Department of Life Sciences, Universidade de Coimbra

Sílvia Castro

FLOWer Lab, Centre for Functional Ecology – Science for People & the Planet, Department of Life Sciences,  
Universidade de Coimbra

Tânia Caetano

CESAM, Departamento de Biologia, Universidade de Aveiro



ORGANIZED BY:



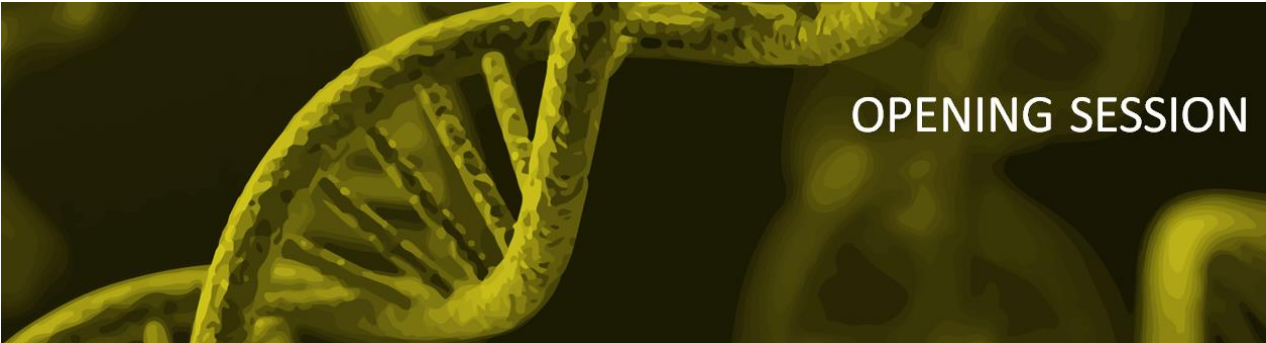
SUPPORTED BY:



SPONSORS







## Miguel Mota and the Portuguese Society of Genetics (SPG)

Manuel G.M. Mota

Departamento de Biologia, Universidade de Évora, 7002-554 Évora, Portugal

Email: [mmota@uevora.pt](mailto:mmota@uevora.pt)

Miguel Eugénio Galvão de Melo e Mota (1922-2016) was an agronomist, cell biologist and geneticist, and a pioneer in plant genetics as well as in plant genetic resources conservation. He was one of the founding members of the “Sociedade Portuguesa de Genética” (SPG) in 1973. Miguel Mota’s interests in Genetics started many years earlier, still as a student of Agronomy, in 1948, and highly influenced by Prof. António Câmara’s classes and inspiring mentorship. This resulted in a study of rabbit genetics. Between 1950-1952, he worked as a post-doc in Albert Levan’s Cytogenetics Lab in the University of Lund, together with fellow Portuguese colleague António Lima-de-Faria. In 1956, in the International Genetics Congress in Japan (Kyoto and Tokyo), he presented “A new hypothesis of the Anaphase movement” which has recently received the well deserved credit. In 1958, the hypothesis was presented in the Xth International Genetics Congress in Montréal. In 1964, a group of Portuguese and Spanish geneticists, participating in the “Simpósio de Genética” in Madrid, organized by Prof. Gimenez-Martinez, decided to implement regular Genetics meetings alternating in both countries. A secretary-general was appointed for each country, Miguel Mota representing Portugal. Thus the “Jornadas de Genética Luso-Espanholas” were inaugurated. In 1965, the first Jornadas was held in Oeiras in the recently inaugurated “Estação Agronómica Nacional” (today, part of INIAV). Except for 1975 and 1988, the Jornadas were held until 1995. The SPG was founded in 1973, following an idea set up during the VIIth Jornadas in Pamplona, in 1970. It’s first President was Amândio Sampaio Tavares (1974-1977). Miguel Mota was President from 2001-2006.



## Fixing heterosis in Brassica crops using apomixis

Timothy Sharbel

Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Room 6E08, Agriculture Building, 51 Campus Drive, Saskatoon, SK S7N 5A8 Canada

Email: [tim.sharbel@usask.ca](mailto:tim.sharbel@usask.ca)

An organism's choice to reproduce with or without sex has long puzzled evolutionary biologists. Apomixis, a natural form of reproduction in plants whereby seeds are produced asexually, has evolved repeatedly from sexual ancestors in many taxa. Apomixis is of interest on a number of levels, ranging from population genetics to evolution, but also from an applied perspective, as it represents a disruptive technology which could significantly change agricultural practices (e.g. fixing heterosis in hybrid crops). The switch from sex to apomixis is hypothesized to result from deregulation of developmental pathways leading to sexual seed development, and the trigger for deregulation involves the global genomic effects of hybridization and polyploidy.

We study apomixis in wild plant populations, and use evolutionary theory to guide our experimental approaches. High-throughput methods are employed to understand population-level phenotypic (seed production) and genetic (polyploidy, genetic structure) variability. These data are then used to design targeted experiments, whereby candidate genes for apomixis are identified using tissue-specific "omics" methods in particular genotypes. These candidates are then used (1) in transformation experiments to attempt apomixis induction in sexual plants, and (2) in population-level studies to understand the origin and evolution of apomixis with respect to sexuality in natural populations. Our goal is to induce apomixis in Brassica crops within the next 5 years.

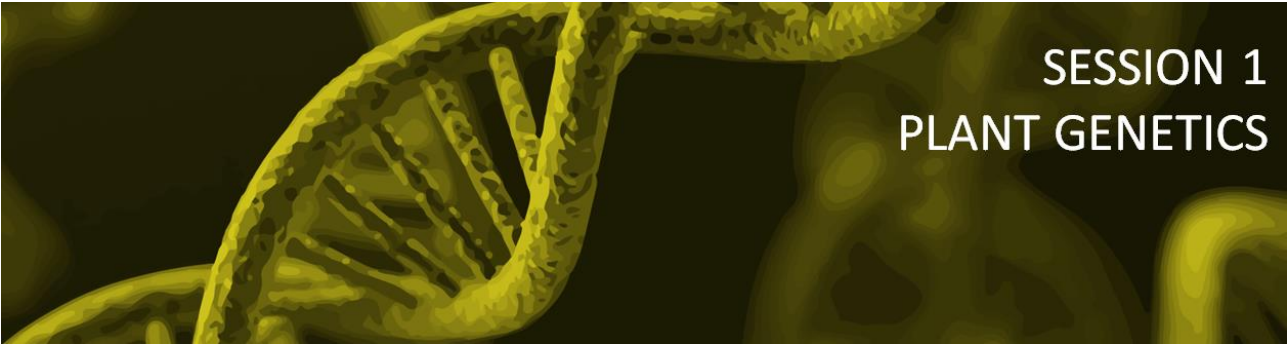
## Understanding and Targeting the TRIB2-AKT-FOXO axis in human cancer and aging

Wolfgang Link

Instituto de Investigaciones Biomédicas “Alberto Sols” (CSIC-UAM), Arturo Duperier 4, 28029-Madri, Spain

Email: [wlink222@gmail.com](mailto:wlink222@gmail.com)

Cancer is the price we pay for longevity. In order to live a long life our cells need to maintain the capacity to proliferate, a process that can run out of control and generate cancer. The insulin signaling pathway has been found to be of paramount importance to both cancer and human longevity. The PI3K/AKT signaling cascade represents a major downstream arm of this nutrient-sensing pathway. Intriguingly, caloric restriction and genetic alterations of components of the PI3K/AKT pathway have been shown to affect longevity in several animal models. FOXO proteins are the major transcriptional effectors of this pathway. FOXO transcription factors are evolutionarily conserved proteins that orchestrate programs of gene expression known to control a variety of cellular processes such as cell cycle, apoptosis, DNA repair and protection from oxidative stress. FOXO factors are context-dependent tumour suppressor proteins commonly inactivated in human tumours and involved in anti-cancer drug resistance. Recent studies have found that FOXO3 is associated with extreme human longevity in Japanese-Americans from Hawaii, Italians, Ashkenazi Jews, Californians, New Englanders, Germans and Han Chinese. We previously developed high-throughput assay systems monitoring FOXO translocation and transcriptional activity to identify chemical compounds and protein targets that interfere with FOXO functions. We have identified several FOXO repressor proteins and FOXO activating compounds. Our objective is to translate our data into clinically useful tools to develop targeted strategies to improve the treatment of cancer as well as human life and healthspan.





## The cis-regulatory code underpinning epigenetic gene regulation

Franziska Turck, Kristin Krause, Simone Zündorf, Maik Mendler, Carina Gude

Max Planck Institute for Plant Breeding Research, Department Plant Developmental Biology, Carl von Linne Weg 10, Köln, 50829, Germany

Presenting author email: [turck@mpipz.mpg.de](mailto:turck@mpipz.mpg.de)

The epigenetic regulatory code sits “on top” of genetic information and helps the genome to select a specific repertoire of expressed genes depending on cell identity and response mode. Histone and DNA-modifications orchestrate the epigenetic code, some promote compacted, inaccessible chromatin states, while others are required to open the chromatin for processes such as gene transcription and DNA-replication. Recent reports have shown that the epigenetic code is heavily underpinned by information at the DNA sequence level and that cognate DNA-binding proteins connect genetic information to and epigenetic regulation. For example, repressive Polycomb Group proteins rely on several DNA-binding proteins that recognize distinct *cis*-regulatory motifs such as TELOMERE REPEAT BINDING FACTORS (TRBs) and VIVIPAROUS AND ABI3 LIKE (VAL) recognizing telo- and RY DNA-motifs, respectively. Interestingly, both motifs are somewhat ambiguous in their effect and can also promote expression of associated genes. My group is interested in understanding how the PcG recruiting *cis*-regulatory code is integrated at target genes and when it evolved within the green lineage

## First initial studies towards the functional characterization of grapevine COP1 homologs

Sara Freitas<sup>1,2,3</sup>, Antonio J. Muñoz-Pajares<sup>1,3,4</sup>, David Azevedo-Silva<sup>1,2,3</sup>, Antero Martins<sup>5,6</sup>, Elsa Gonçalves<sup>5,6</sup>, Mariana Sottomayor<sup>1,2,3</sup>, Miguel Carneiro<sup>1,2,3</sup>, Pedro Humberto Castro<sup>1,3</sup>, Herlander Azevedo<sup>1,2,3</sup>

<sup>1</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal <sup>3</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

<sup>4</sup>Departamento de Genética, Facultad de Ciencias, Universidad de Granada. Campus Fuentenueva, 18071, Granada, Spain

<sup>5</sup>LEAF- Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>6</sup>Portuguese Association for Grapevine Diversity-PORVID, Tapada da Ajuda, 1349-017 Lisboa, Portugal

Email: [hazevedo@cibio.up.pt](mailto:hazevedo@cibio.up.pt)

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated and economically significant crops in the world, with 7.5 mha of worldwide cultivated area generating 259 mhl of wine production. To explore the diversity encased in the valuable Portuguese grapevine germoplasm we conducted a Pool-Seq genomics strategy, and subsequently generated genome-level Fst data that enables selective sweep mapping for traits of interest. Analysis of selective sweep presence for the trait berry colour suggested the involvement of a grapevine CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) homolog. COPs are RING-finger proteins that act as central switches of light-responsive developmental and physiological processes, and are central repressors of the MBW complex (the master regulator of anthocyanin biosynthesis)<sup>1</sup>. Considering the lack of knowledge on grapevine COP1 homologs, we initiated follow up studies towards a better understanding of these proteins in grapevine. Initial efforts include a phylogenetic and comparative genomics analysis of COP1 homologs, the disambiguation of family member number across the multiple *Vitis vinifera* genome annotations, and gene expression profiling using publicly available transcriptomics data. We also initiated studies towards addressing the more functional aspects of grapevine COP1 homologs.

This work was financed through Fundação para a Ciência e Tecnologia (FCT/MCTES) for project GrapeVision (PTDC/BIA-FBT/2389/2020) and support to H.A. (CEECIND/00399/2017/CP1423/CT0004); FCT/MCTES and POCH/NORTE2020/FSE for support to S.F. (SFRH/BD/120020/2016); FCT/MCTES and POPH-QREN/FSE for support to M.C. (CEECINST/00014/2018/CP1512/CT0002).

### References

<sup>1</sup>LaFountain and Yuan (2021). *New Phytologist*, 231, 933–949. doi: 10.1111/nph.17397.

***Citrus clementine* and *Citrus sinensis* callose synthase and xyloglucan endotransglucosylase gene expression along time in response to *Citrus tristeza virus* infection**

Melina Silva<sup>a,c</sup>, Sandra Germano<sup>b</sup>, Patrícia Pinto<sup>c</sup>, Amílcar Duarte<sup>d</sup>, Deborah M. Power<sup>c</sup>, [Natália T. Marques<sup>d</sup>](mailto:nmarques@ualg.pt)

<sup>a</sup>Center of Electronics, Optoelectronics and Telecommunications, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>b</sup>Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal

<sup>c</sup>Comparative Molecular and Integrative Biology Group, Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>d</sup>Centre for Mediterranean Bioresources and Food (MeditBio), Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Email: [nmarques@ualg.pt](mailto:nmarques@ualg.pt)

The invasion of plant cells by a virus triggers numerous defensive proteins to block progression inside the host. Callose deposition on plasmodesmata and phloem sieve plates occurs minutes after pathogen infection generate a physical barrier to reduce or impede its movement to uninfected cells. *Citrus tristeza virus* (CTV) is a phloem-confined virus and the causal agent of Tristeza disease, one of the more destructive citrus diseases. Upon CTV infection, transcripts of enzymes involved in callose deposition such as callose synthase, hemicellulose synthesis, and xyloglucan endotransglucosylase, are up-regulated in infected plants. In the present study, transcripts of callose synthase 7 isoform 5 (CaIS7x5) and xyloglucan endotransglucosylase 9 (XHT9) were evaluated by quantitative(q) PCR in *Citrus sinensis* cv. Valencia late (VL) and *Citrus × clementina* ‘Fina’ (CL) stem tissues, infected with a severe CTV isolate, T36. qPCR analysis of healthy and infected citrus was performed at 15 days post-inoculation, 10 months post-inoculation (mpi) and at 31 mpi with CTV. The CTV titer increased along time in young bark tissues of both citrus species, with VL plants exhibiting a titer about 5 times higher than CL at 31 mpi. *CaIS7x5* gene was usually underexpressed in both citrus species infected with CTV but levels were not significantly different from control plants. Distinct and significant impacts of infection were found on *xth9* expression for both citrus species after 31 mpi. In CL, infected with CTV, *xth9* was down regulated, while VL infected plants had significantly increased *xth9* gene expression at 31 mpi compared to its controls. These results suggest the two citrus varieties had a different response to CTV, possibly due to differing susceptibility, since VL had a viral titer 4.9 times higher than CL.

## Gene expression associated to essential oil production in different ploidal levels of *lippia alba*

Juliana Mainenti Leal Lopes<sup>1,2,3</sup>, Laís Stehling de Queiroz Nascimento<sup>1</sup>, Vinicius Carius Souza<sup>1</sup>, Elyabe Monteiro de Matos<sup>1</sup>, Evandro Fortini<sup>4</sup>, Richard Grazul<sup>5</sup>, Marcelo Oliveira Santos<sup>1</sup>, Douglas E. Soltis<sup>6,7</sup>, Pamela S. Soltis<sup>6</sup>, Wagner Campos Otoni<sup>4</sup>, Lyderson Facio Viccini<sup>1</sup>

<sup>1</sup>Departamento de Biologia, Instituto de Ciências Biológicas, UFJF, Juiz de Fora, Minas Gerais, 36036-900, Brazil

<sup>2</sup>School of Life Science and Environment, Department of Genetic and Biotechnology, UTAD, 5001-801 Vila Real, Portugal

<sup>3</sup>BiolSI, University of Lisboa, 1649-004 Lisboa, Portugal

<sup>4</sup>Laboratório de Cultura de Tecidos Vegetais (LCTII), Departamento de Biologia Vegetal/BIOAGRO, UFV, Av. P.H. Rolfs s/n, Campus Universitário, 36570-000 Viçosa, MG, Brazil

<sup>5</sup>Department of Chemistry, UFJF, Juiz de Fora, Brazil

<sup>6</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA

<sup>7</sup>Department of Biology, University of Florida, Gainesville, FL 32611, USA

Email: [julianamainenti@gmail.com](mailto:julianamainenti@gmail.com)

*Lippia alba* (Mill.) N. E. Brown (Verbenaceae) is widely used in popular medicine for sedative, analgesic, anti-inflammatory, anti-hypertensive, and antifungal purposes<sup>1</sup>, due to the composition of its essential oils. Monoterpenes are the main component of essential oils of *Lippia alba* and the chemical composition of them varies with the genome size: citral (geraniol and neral) is dominant in diploids and tetraploids while linalool is the main component in triploids<sup>2,3</sup>. Considering that environmental stress impacts various metabolic pathways, we hypothesized that stress responses in *L. alba* could regulate the relationship between genome size and essential oil composition. Water stress affects flowering, production, and reproduction of angiosperms. Here, we evaluated the effect of water stress, whereby 1% PEG-4000 was added to MS medium, on essential oil production, and the expression of genes related to monoterpene synthesis in diploid, triploid, and tetraploid accessions of *L. alba* cultivated *in vitro* for 40 days. First, using transcriptome data, we performed *de novo* gene assembly and identified orthologous genes using phylogenetic and clustering-based approaches. Next, we assessed the expression of these genes by real-time quantitative PCR under water stress conditions. Gene expression was related to essential oil production in triploid accessions. The changes in production of essential oils show the complex regulation of metabolic pathways in polyploid genomes. In addition, they highlight aspects of genotype and environment interactions, which may be important for the understanding of the origin and conservation of tropical biodiversity.

This work was financed through the projects CAPES, CNPq and FAPEMIG.

### References

- <sup>1</sup>Cunha MA, Barros FMC, Garcia LO, Veeck APL, Heinzmann BM, Loro VL, Emanuelli T, Baldisserotto B (2010). Aquac. Res. 306 403-406.
- <sup>2</sup>Viccini LF, Silveira RS, Do Vale AA, De Campos JMS, Reis AC, De Oliveira Santos M, Grazul RM (2014) Ind Crops Prod 59, 14–19.
- <sup>3</sup>Lopes JML, Carvalho HH, Zorzatto C, Azevedo AL, Machado MA, Salimena FRG, Grazul RM, Gitzendanner MA, Soltis DE, Soltis PS, Viccini IF (2020) Am. J. Bot.107(3): 466-476.

## **A genome-wide analysis of *Mitochondrial Uncoupling Proteins (UCP)* gene family in *Olea europaea* L. reveals a diversity of transcript patterns during adventitious root formation**

Cristina Mendes<sup>1</sup>, [Lénia Rodrigues](#)<sup>2</sup>, Augusto Peixe<sup>3</sup>, Hélia Cardoso<sup>2</sup>

<sup>1</sup>Escola de Ciências e Tecnologia, Universidade de Évora, Lab. Biologia Molecular, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

<sup>2</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, IIFA - Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

The development of adventitious roots (AR) on stem cuttings is considered a morphological response to stress, being dependent of a great number of internal and external factors. Mitochondria play a central role in plant cell response to abiotic stresses and reacquisition of metabolic homeostasis, being some proteins located at the inner mitochondrial membrane known as involved on those processes. On this study, olive microcuttings already cultured *in vitro* were used to evaluate the possible involvement of *Mitochondrial Uncoupling Proteins (UCP)* gene family members on olive AR formation. Explants treated for root induction with IBA (pulse for 10 seconds on a 3000 ppm IBA solution) were compared with non-treated ones (control samples). Biological samples, consisting of the bases of the microcuttings, were collected from 0h to 17 days on both groups. After RNA extraction and cDNA synthesis, gene expression was evaluated by Reverse Transcription quantitative PCR (RT-qPCR) in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). *Ubiquitin* and *Histone2B* were used as reference genes for target genes normalization. Phylogenetic analysis revealed the existence of four *OeUCPs* genes, named *OeUCP1*, *OeUCP2*, *OeUCP3* and *OeUCP5*. All genes exhibited changes in transcript accumulation during the experiment time course. However, only *OeUCP5* presented a significant difference between IBA-treated and non-treated explants, seen at the early timepoint (4h post IBA treatment). The understanding of the molecular mechanisms involved in the induction of adventitious roots may help to develop more effective rooting induction protocols and improve the rooting ability of difficult-to-root cultivars.

Authors would thank to Virginia Sobral by the help on the establishment of the *in vitro* trials. This work is supported by National Funds through FCT under the Project UID/AGR/00115/2019 and co-funded by FEDER through ALENTEJO 2020 under ALT20-03-0145-FEDER-000014.

## Identification and characterization of Histone acetylation associated proteins in Fagaceae

Sofia Alves<sup>1</sup>, Ângelo Braga<sup>2</sup>, Denise Parreira<sup>2</sup>, Ana Teresa Alinho<sup>3</sup>, Miguel Ramos<sup>1</sup>, Maria Manuela R. Costa<sup>3</sup>, Leonor Morais-Cecílio<sup>1</sup>

<sup>1</sup>LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>2</sup>Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>3</sup>Centre of Molecular and Environmental Biology (CBMA), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Email: [salves@isa.ulisboa.pt](mailto:salves@isa.ulisboa.pt)

Fagaceae is a family of trees and shrubs of the Northern hemisphere, which includes oaks, chestnuts, and beeches. These trees have a fundamental ecological role in their habitats and a high economic importance due to the value of its fruits and wood. Epigenetic regulation is a process by which gene expression is modulated, important for plant survival and reproduction like bud dormancy/break and flower development. Histone acetyltransferases (HATs) and deacetylases (HDACs) are epigenetic regulators that change the acetylation status of histones tails residues, rendering the genes associated to that region transcriptional active or silenced, respectively. To characterize these proteins in different species of Fagaceae, free access RNA-seq information was used to assemble *de novo* transcriptomes of *Castanea* (*C. sativa*, and *C. henryi*) and *Fagus* (*F. grandifolia*). Together with the published genomes from *F. sylvatica*, *F. crenata*, *Quercus suber*, *Q. robur*, *Q. lobata*, *C. crenata*, *C. dentata* and *C. mollissima*, we were able to identify six HATs and eleven HDACs orthologs of *Arabidopsis thaliana*, with their predicted domains. The presence of paralogs was evaluated for each species. HDACs show more gene duplication than HATs, with more duplications observed in *Quercus* and *Castanea*. Phylogenetic trees using Maximum Likelihood as optimization criteria were created using the protein sequences of all Fagaceae and *A. thaliana*. The generated trees individualize gene clusters according to the expected phylogenetic relationships supported by good bootstrap values, with the species clustered by genus. As the histone acetylation process is still poorly understood in Fagaceae, this study facilitates further studies concerning epigenetic regulation of critical developmental processes and environmental responses in this economically important family.

## A link between the SUMO E3 ligase SIZ1 and reactive oxygen species homeostasis in *Arabidopsis thaliana*

Pedro Humberto Castro<sup>1,2</sup>, Daniel Couto<sup>3</sup>, Miguel Ângelo Santos<sup>3</sup>, Sara Freitas<sup>1,2,3</sup>, Tiago Lourenço<sup>1,2,3</sup>, Eva Dias<sup>3</sup>, Stéphanie Huguette<sup>4</sup>, Jorge Marques da Silva<sup>5</sup>, Rui Manuel Tavares<sup>3</sup>, Eduardo Rodríguez Bejarano<sup>7</sup>, Herlander Azevedo<sup>1,2,8</sup>

<sup>1</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>2</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

<sup>3</sup>Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

<sup>4</sup>Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91405, Orsay, France

<sup>5</sup>Université de Paris, CNRS, INRAE, Institute of Plant Sciences Paris-Saclay (IPS2), 91405, Orsay, France

<sup>6</sup>Biosystems and Integrative Sciences Institute (BioISI) and Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

<sup>7</sup>Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora”, Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Dept. Biología Celular, Genética y Fisiología, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain

<sup>8</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

Email: pedro1berto@cibio.up.pt; [hazevedo@cibio.up.pt](mailto:hazevedo@cibio.up.pt)

The peptide SUMO has become a recognised regulator of the plant response to multiple environmental stresses. These external stresses normally induce the production of reactive oxygen species (ROS). Taking into account that SUMO-conjugates rapidly accumulate in response to an external oxidative stimulus, it is likely that ROS and sumoylation converge at the molecular and regulatory levels. In this recently published work<sup>1</sup>, we explored the SUMO-ROS relationship, using as a model the *Arabidopsis* null mutant of the E3 ligase SIZ1 known as the major SUMO-conjugation enhancer in plants. We showed that SIZ1 is involved in SUMO-conjugate increment when primed with both exogenous and endogenous ROS. The *siz1* seedlings were sensitive to oxidative stress imposition and accumulated different ROS throughout development. We demonstrated that the deregulation in hydrogen peroxide and superoxide homeostasis, but not of singlet oxygen, was partially due to salicylic acid accumulation in *siz1*. In addition, we observed that *siz1* displayed chloroplast morphological defects, altered energy dissipation activity, and accumulation of the chlorophyll precursor PChlide via downregulation of *PORA*, which drives overproduction of singlet oxygen. Finally, network analysis uncovered known and new associations between transcriptional control of *PORA* and SIZ1-dependent sumoylation, important to the control of ROS homeostasis and signalling events.

### References

<sup>1</sup>Castro PH et al. (2022) *Plant Physiology* (In press). DOI 10.1093/plphys/kiac085

## Assessment of Downy Mildew Resistance and Genetic Relationships Among Wild Rocket (*Diplotaxis tenuifolia* (L.) DC) Accessions

Paula Coelho<sup>1</sup>, [João Reis](#)<sup>2</sup>, Ana Pereira<sup>1</sup>, Aliana Vairinhos<sup>2</sup>, Violeta Lopes<sup>3</sup>, José Leitão<sup>2</sup>

<sup>1</sup>INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, Av. da República, 2784-505 Oeiras, Portugal

<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>3</sup>BPGV/INIAV - Banco Português de Germoplasma Vegetal, Polo de Inovação de Braga, 4700-859 Braga, Portugal

Email: [jleitao@ualg.pt](mailto:jleitao@ualg.pt), [paula.coelho@iniav.pt](mailto:paula.coelho@iniav.pt), [a61179@ualg.pt](mailto:a61179@ualg.pt)

Wild rocket (*Diplotaxis tenuifolia* (L.) DC.) is an herbaceous species produced as baby leaf salad, which importance is increasing as a response to the growing preference of the consumers over the “cultivated” rocket (*Eruca* spp.). Downy mildew (DM) disease, elicited by the oomycete *Hyaloperonospora* sp., is one of the main causes of yield losses in wild rocket cultivation. From a point of view of food safety, environmental concerns and viability of organic farming production the identification of resistant genotypes is a priority to this industry. A set of 29 *D. tenuifolia* accessions, and 1 *Eruca* spp. accession, were screened at the seedling stage, for resistance to the *Hyaloperonospora* sp. isolate D5, collected on *D. tenuifolia* infected plants. The plant/pathogen interaction phenotype (IP) was assessed at the cotyledon and first two leaves stage, and the accessions were classified as resistant (R), partially resistant (PR), susceptible (S) and highly susceptible (HS) in both stages. The *Eruca* spp. accession was resistant at both stages (R/R), while the *Diplotaxis* accessions were, respectively, 1 (R/R), 3 (PR/R), 8 (S/PR), 6 (HS/PR), 9 (HS/PR) and 2 (HS/HS). A significant correlation ( $r=0,865896$ ,  $P<0.001$ ) was found between the disease index values exhibited in cotyledons vs. young leaves. No plants R or PR in cotyledons became susceptible in the first leaves. Molecular analyses were performed using 7 RAPD and 5 ISSR markers, which have amplified 110 clearly scorable markers. As expected for genotypes from the same species, the genetic similarity among the *Diplotaxis* accessions varied from 0,751 to 0,994. The *Eruca* accession exhibited very low genetic similarity (0,171) to the wild rocket accessions. Some of the accessions that exhibited high genetic similarity were provided by the same breeding company.

This work was funded by the project: PTDC/ASP-PLA/28963/2017- REMIRucula - Resistance characterization to downy mildew in wild rocket crop.



## Bud dormancy and flowering regulation mechanisms in *Castanea sativa* Mill.

Ana Teresa Alinho<sup>1</sup>, José Gomes-Laranjo<sup>2</sup>, Rómulo Sobral<sup>1</sup>, Maria Manuela Ribeiro Costa<sup>1</sup>

<sup>1</sup>Centre for Molecular and Environmental Biology (CBMA), University of Minho, Campus de Gualtar,

4710-057 Braga, Portugal

<sup>2</sup>Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Universidade of Trás os Montes and Alto Douro, Quinta de Prados, 5000-801, Vila Real, Portugal

Email: [b7682@bio.uminho.pt](mailto:b7682@bio.uminho.pt)

The sweet chestnut tree (*Castanea sativa* Mill.) is one of the most significant Mediterranean tree species, being an important natural resource for the wood and fruit industries. During the span of one year, *C. sativa* trees go through several developmental transitions: in fall, entrance into dormancy occurs concomitantly with the shortening of day length and decrease in temperature, and dormancy break in spring is followed by vegetative and reproductive growth. Regarding flowering, it is a monoecious tree with accentuated proterandry: in spring, unisexual male catkins emerge in the axils of new leaves, whereas bisexual catkins emerge one month later in the terminal end of the shoots.

The development of flowers in summer is dependent on several genetic pathways that signal the plant the appropriate timing for flowering, and are well known in some model herbaceous species. This signalling is tightly regulated and synchronised by local climate conditions, such as temperature and photoperiod. Despite the overall importance of this species, the molecular mechanisms that control its flower induction and development, and its dormancy transitions, are still elusive.

In the present study, phylogenetic profiling lead to the identification of *C. sativa* homologs of key floral regulatory genes, and the expression profile of these flowering regulators was assessed during several seasons. The results suggest that flowers may be induced during summer, staying enclosed inside the dormant buds until the following year, displaying a long anthesis period. Through a transcriptomics approach, transcript expression patterns allowed the identification of potential target genes associated with dormancy establishment and dormancy break in this species.

Overall, the results here presented constitute a step forward in the elucidation of the molecular mechanisms responsible for the different stages of *C. sativa* yearly cycle. This knowledge may be introduced in orchard management, thus improving profitability.

This work was supported by CBMA through the “Contrato-Programa” UIDB/04050/2020, and by POCI-01-0145-FEDER-027980/PTDC/ASP-SIL/27980/2017, by national funds through the FCT I.P. A.T.A was funded by the Ph.D. grant SFRH/BD/136834/2018.

## Characterization of microRNAs implicated in maritime pine resistance to pinewood nematode

André Mendes<sup>1,2</sup>, Inês Modesto<sup>3</sup>, Ana Milhinhos<sup>1,3</sup>, Célia Miguel<sup>1</sup>

<sup>1</sup>Faculdade de Ciências, Biosystems & Integrative Sciences Institute, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal

<sup>2</sup>Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal

<sup>3</sup>GREEN-IT-ITQB-NOVA-Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

Email: [mrandremendes@gmail.com](mailto:mrandremendes@gmail.com)

Pine wilt disease (PWD), caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, is one of the major threats to conifer forests worldwide<sup>1</sup>. In Europe, since 1999, PWD has spread from Portugal to the Madeira Island and Spain<sup>1</sup>. The most affected European species is the maritime pine (*Pinus pinaster*) — the third most represented forest species in Portugal, occupying close to 30% of the forest area and accounting for 80% of the employment in the forestry sector<sup>2</sup>. Therefore, significant efforts to identify putative plant resistance mechanisms have been conducted.

In this work, microRNAs (miRNAs) from *P. pinaster* genotypes belonging to previously identified half-sib families showing variable degrees of resistance to PWN<sup>3</sup> are being characterized by quantifying their expression levels and of their predicted targets at 0, 24, 48 and 72h post-inoculation. The selected miRNAs have been found to be differentially expressed in resistant *versus* susceptible genotypes<sup>4</sup>. Their predicted targets suggest involvement in the regulation of plant defense and resistance to PWN, such as in jasmonate-response pathway, ROS detoxification, and terpenoid biosynthesis<sup>4</sup>. The current research has identified suitable miRNAs to be used as reference transcripts for a more reliable miRNA expression normalization by RT-qPCR in PWD experiments. The miRNAs quantification has disclosed differential expression tendencies across the different time-points and between resistant, susceptible and non-inoculated genotypes. Furthermore, promising ongoing metabolite profiling assays seem to suggest differential terpene expression among those genotypes.

The integration of these results will hopefully clarify the implication of the studied miRNAs on *P. pinaster*' PWN-resistance traits. This is an important step towards the development of future breeding strategies for *P. pinaster*, aiming to mitigate the spread of PWD and its devastating consequences.

### References

- <sup>1</sup>Vicente C, Espada M, Vieira P, Mota M (2012). Pine Wilt Disease: A threat to European forestry. *European Journal of Plant Pathology*, 133(1), 89-99.
- <sup>2</sup>Centro PINUS (2021). A Fileira Do Pinho Em 2020.
- <sup>3</sup>Carrasquinho I, Lisboa A, Inácio ML, Gonçalves E (2018). Genetic variation in susceptibility to pine wilt disease of maritime pine (*Pinus pinaster* Aiton) half-sib families. *Annals of Forest Science*, 75(3), 1-11.
- <sup>4</sup>Modesto I, Inácio V, Van de Peer Y, Miguel CM (2022). MicroRNA-mediated post-transcriptional regulation of *Pinus pinaster* response and resistance to pinewood nematode. *Scientific Reports*, 12(1), 5160.

## Detection of Natural Hybrids between *Quercus suber* L. and *Q. rotundifolia* Lam. in Portugal

Davide Botticelli, Carlos Vila-Viçosa, Paulo Oliveira, Herlander Azevedo, Mariana Sottomayor, Pedro Esteves, [Ana Campilho](mailto:anacampilho@bio.up.pt)

CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

Email: [anacampilho@bio.up.pt](mailto:anacampilho@bio.up.pt)

*Quercus suber* and *Quercus rotundifolia* are two Mediterranean evergreen oaks with a strong presence in the Portuguese landscape, especially in the typical Montado ecosystems of Southern Portugal (Alentejo and Ribatejo), where natural hybrids (*Q. × avellaniformis* Colmeiro & E. Boutelou) can be found. The aim of this work was to use genetic markers to identify putative hybrids previously referenced in the Portuguese territory by their morphological characteristics. Hence, leaf samples were collected from 30-40 individuals of each *Q. suber* and *Q. rotundifolia*, at different locations in North, Center and South Portugal, as well as from individuals of putative *Q. × avellaniformis*. After genomic DNA extraction, the individuals were genotyped with eight previously reported microsatellite loci, followed by a Bayesian clustering analysis implemented in STRUCTURE version 2.3.4 software. Independent runs with values of K (i.e. potential number of genetic clusters) ranging from 1 to 4 were performed for the dataset. The simulations with K=2 perfectly defined two genetic clusters that effectively corresponded to the two *Quercus* species. Most of the putative hybrids showed a contribution from both genetic pools. In conclusion, the method proved to be reliable for the molecular identification of the hybrids *Q. × avellaniformis*.

Work was funded by FCT.

## Differential gene expression in wound and auxin-related responses in *Prunus dulcis* Mill. micrografting

Sandra Caeiro<sup>1</sup>, Tércia Lopes<sup>2</sup>, Ana Pedrosa<sup>2</sup>, Rita Costa Pires<sup>1</sup>, Ana Faustino<sup>1,3</sup>, Jorge Canhoto<sup>2</sup>, Liliana Marum<sup>1,3</sup>, Sandra Correia<sup>2,4</sup>

<sup>1</sup>Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, PORTUGAL

<sup>2</sup>University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, PORTUGAL

<sup>3</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801- 908, Beja, Portugal

<sup>4</sup>InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

Email: [sandra.caeiro@cebal.pt](mailto:sandra.caeiro@cebal.pt)

In recent years the increasing demand for dry fruits led to a higher demand and consumption of almond. During grafting, the propagation method most widely used for *Prunus dulcis* Mill., the transport dynamics are affected. However, once the scion and the rootstock join, a complex process involving cell division and differentiation of vascular tissues establishes communication between the two parts. Nevertheless, the regulatory mechanisms involved in scion-rootstock interactions remain largely unknown<sup>1,2</sup>. The aim of this work was to study wound healing and auxin-induced regulatory mechanisms involved in scion-rootstock interactions in almond micrografts. To achieve this goal, it was necessary to establish and multiply *in vitro* the almond plant material. Then, micrografting was performed, establishing homografts and heterografts. Gene expression quantification of a wound-related AP2/ERF transcription factor WOUND INDUCED DEDIFFERENTIATION 1 (*WIND1*) and of genes involved in auxin responses to promote the vascular connection between the scion and the rootstock (*ALF4*, *TIR1* and *IAA26*) was carried out before micrografting (T0), 7 days after micrografting (T1) and 21 days after micrografting (T2).

In the results here obtained, homografts showed a possible simultaneous wound and auxin response, while heterografts showed a possible earlier wound response and the absence of a proper auxin response at the graft union.

This work was made in the scope of CULTIVAR project (CENTRO-01-0145-FEDER-000020), co-financed by the Regional Operational Programme Centro 2020, Portugal 2020 and European Union, through European Fund for Regional Development (ERDF); Inov-Amendo-AL: Microenxertia in vitro de amendoeiras selecionadas para a promoção do amendoal no Alentejo (ALT20-03-0246-FEDER-000068) supported by Program Alentejo 2020, through the European Fund for Regional Development (ERDF), within the scope of the Collective Action Support System - Transfer of scientific and technological knowledge. Domain of Competitiveness and Internationalization. Authors also acknowledge Centre for Functional Ecology, FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018) and FCT for UIDB/05183/2020, FCT for Contrato—Programa to L.Marum and LA/P/0121/2020 projects.

### References

- <sup>1</sup>Queirós, F. (2020). Manual de boas práticas de fruticultura. 5ª fascículo: Amendoeira. Frutas Legumes e Flores in collaboration with INIAV, I.P. (Estação Nacional de Fruticultura Vieira Natividade) and COTR.
- <sup>2</sup>Melnyk, C. W. (2015). Plant grafting: insights into tissue regeneration. *Regeneration*, 4(1), 3–14.

## Exploring plant metabolomics for the control of the root-lesion nematode *Pratylenchus penetrans*

Marina Costa<sup>1</sup>, Jorge Faria<sup>1</sup>, Dora Teixeira<sup>2</sup>, Pedro Barbosa<sup>3</sup>, Margarida Espada<sup>3</sup>, Manuel Mota<sup>4</sup>, Maria de Lurdes Inácio<sup>1</sup>, Cláudia Vicente<sup>3</sup>

<sup>1</sup>Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal.

<sup>2</sup>HERCULES Laboratory, Institute for Advanced Studies and Research, University of Évora, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies, and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>4</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Biology, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [cvicente@uevora.pt](mailto:cvicente@uevora.pt)

Plant-parasitic nematodes (PPN) are a major threat to global agriculture with annual losses estimated at \$78-125 billion<sup>1</sup>. Present restrictions on the use of synthetic chemical pesticides, harmful for both human health and environment, impose the re-evaluation of the control strategies and the investment in novel and sustainable approaches to mitigate the damage caused by PPN. The root-lesion nematode (RLN) *Pratylenchus penetrans* is one of the most detrimental RLNs, greatly reducing the production of numerous important food and feed crops<sup>2</sup>. This RLN has been classified as an A1 quarantine pest in South America in 2018, while in the European Union, it was recently listed as a regulated A2 non-quarantine pest. In Europe, *P. penetrans* has been reported as one of the most damaging species associated with potato (*Solanum tuberosum* L.)<sup>3,4</sup>. Plant metabolomics is an emerging approach to the study of crop resistance in response to PPN infection, which can be applied to accelerate traditional crop breeding programs and for the development of novel pesticides. For this purpose, the project PratyOmics aims: (i) to compare global metabolomic profiling of resistant and susceptible potato cultivars in response to *P. penetrans* infection for the identification of host resistant-induced plant secondary metabolites (PSM) with potential anti-nematode activity; (ii) to evaluate the nematicidal bioactivity of the candidate PSM against *P. penetrans*; and (iii) to understand the mechanism of action of the most promising PSM by transcription profiling of nematode affected molecular pathways, which can be translated into targets for the development of new effective control strategies.

### References

<sup>1</sup>Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockand S, Tahna Maafi Z (2011) *Genomics and molecular genetics of plant-nematode interactions*. Springer, Dordrecht, pages 21–43.

<sup>2</sup>Vicente CSL, Inácio ML, Mota M, Vieira P (2021) O nemátode das lesões radiculares. *Vida Rural*, Abril, 78-82.

<sup>3</sup>Orlando V, Grove IG, Edwards SG, Prior T, Roberts D, Neilson R, Back M (2020) *Plant Pathology* 69:405-417.

<sup>4</sup>Abrantes IMO, Faria CAT, Santos MSN. (1987) *Nematologia Mediterranea* 15: 375-378.

## Exploring the function of iodine regulatory gene families in plants

Tiago Lourenço<sup>1,2,3,4\*</sup>, Alexandre Esperança<sup>1,2,3</sup>, Sara Freitas<sup>1,2,3</sup>, Christian Dubos<sup>4</sup>, Herlander Azevedo<sup>1,2,3</sup>, Pedro Humberto Castro<sup>1,2\*\*</sup>

<sup>1</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>2</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

<sup>3</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

<sup>4</sup>BPMP, Université de Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France.

\*Presenting author email: [tiagolourenco@cibio.up.pt](mailto:tiagolourenco@cibio.up.pt); \*\*Correspondence: [pedro1berto@cibio.up.pt](mailto:pedro1berto@cibio.up.pt)

Iodine is a scarce yet crucial element in the human diet. Iodine is involved in human neurological and physical development by acting as a key component of thyroid hormones. Insufficient iodine intake results in various Iodine Deficiency Disorders (IDDs), which affect billions of people. A relevant strategy to increase iodine levels in humans include the prophylactic consumption of iodized salt, but additional and combinatory strategies are necessary to successfully mitigate this global problem. Recently, the development of iodine biofortified crops has been discussed as a promising strategy to combat IDDs. In plants, iodine is considered a beneficial element for plant growth and the response to environmental stress. Regardless of its importance, assimilation and mode-of-action of iodine at the plant molecular level are still not understood. Based on a comparative genomics strategy, we have established several parallelisms for iodine regulation between plants and non-plant organisms. Here, we will explore a gene family involved in the homeostatic control of iodine in plants. A phylogenetic overview will highlight the degree of conservation of this gene family in plants. To explore the importance of these genes in iodine content regulation, Arabidopsis T-DNA insertion mutants and overexpression lines will be established for functional genomics analysis. A developmental map of these lines will be performed and Arabidopsis seedlings will be subjected to varying concentrations of iodine. The impact of these treatments will be studied through the analysis of morphology/physiology parameters and the iodine content quantification will be indicative of the thresholds for iodine biofortification and tolerance in plants.

The work was supported by FCT/MCTES, FEDER and COMPETE/POCI (PTDC/BAA-AGR/31122/2017, POCI-01-0145-FEDER-031122) and BIOPOLIS - European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement Number 857251). T.L. was supported by FCT/MCTES, NORTE2020 and UE/FSE (SFRH/BD/05206/2021).

## Exploring the involvement of *Alternative oxidase 1a* on somatic embryogenesis in *Olea europaea*

Diogo Marques<sup>1</sup>, Lénia Rodrigues<sup>2</sup>, Rita Pires<sup>2</sup>, Augusto Peixe<sup>3</sup>, Hélia Cardoso<sup>2</sup>

<sup>1</sup>Escola de Ciências e Tecnologia (ECT), Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, IIFA - Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

<sup>3</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, ECT, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

The alternative oxidase (AOX) enzyme is known to be induced in response to several biotic and abiotic stress conditions, and previous studies have suggested a correlation between AOX and cellular reprogramming through the embryogenic pathway<sup>1</sup>. To test the involvement of AOX subfamily-1a member (*OeAOX1a*) during the expression phase of somatic embryogenesis in olive, two embryogenic cell lines from the cv. 'Galega vulgar' with different embryogenic competence were selected. Both cell lines were subjected to a heat stress treatment (hts) (40°C for 4h) being further transferred to 25°C. Samples were collected at 0h (previously to hst), immediately after hts, and at 24h, 6 days and 12 days after hst. Control samples consisted of the two cell lines maintained upon 25°C during all experiment time course. The involvement of *OeAOX1a* was evaluated at the transcript level by RT-qPCR, and at the protein level by Western blot. The low-performance cell line exhibited higher *OeAOX1a* transcript accumulation, being differences also observed when the results achieved at 40°C and 25°C were compared. The expression peak was observed immediately after heat stress treatment, with the levels tending for the initial transcript over time. The results related with the protein level exhibited the same pattern as those obtained for the transcript. The positive effect of the heat treatment on embryo differentiation was visible on the high-performance embryogenic cell line, with a higher number of differentiated embryos when compared with the control sample. The obtained results demonstrate the involvement of *OeAOX1a* on olive SE efficiency and the positive effect of the heat treatment on the process.

### References

<sup>1</sup>Arnholdt-Schmitt B, Ragonazi C, Cardoso H (2016) Do Mitochondria play a central role in stress-induced somatic embryogenesis? In "in vitro embryogenesis in higher plants", M. A. German, M. Lombardi (eds.); Vol 1359 of the Methods in Molecular Biology: Springer New York. ISBN: 9781493930609

## Exploring the involvement of the somatic embryogenesis receptor kinase (SERK) gene family in olive somatic embryogenesis efficiency

Rita Pires<sup>1</sup>, Diogo Marques<sup>2</sup>, Catarina Campos<sup>1</sup>, Augusto Peixe<sup>3</sup>, Hélia Cardoso<sup>1</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Formação e Investigação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal

<sup>2</sup>Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [rnpires@uevora.pt](mailto:rnpires@uevora.pt), [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

Several Portuguese olive tree cultivars are characterized by their high-quality oils, which explains their use on the production of DOP (Denomination of Protect Origin) olive oil brands. However, some of those cultivars are affected by serious agronomic problems, including high susceptibility to biotic and abiotic stresses, excessive vigour, and low ability to adventitious root formation, which strongly limits its propagation by cuttings. The establishment of somatic embryogenesis (SE) protocols can work as an alternative for propagation of those cultivars. Nevertheless, the efficiency of SE strongly depends on the complex interaction between numerous factors, including amongst others the genotype of the donor plant, the type of explant used to establish the *in vitro* cultures and its developmental stage. Plant regeneration through SE involves changes in gene expression and has been associated with changes in DNA methylation in several model plants, but little is known about the genes expressed during SE in olive. Somatic embryogenesis receptor kinase (SERK) belongs to a small family of receptor-like kinases involved in signal transduction in various plant species. In the present research, the involvement of SERK members in SE efficiency was evaluated by using two olive somatic embryogenic lines, characterized by their different ability to differentiate somatic embryos (high and low-efficient lines). *In vitro* cultured *calli* from both lines were stimulated to undergo cyclic embryogenesis by inoculation on Olive Cyclic Embryogenic liquid medium. Samples from the cell lines were collected at 7 days and further used for total RNA extraction and cDNA synthesis. The involvement of SERK gene family members was evaluated by RT-qPCR and the results achieved revealed a different regulation when comparing high and low efficient cell lines. Differences between timepoints were also detected. The involvement of epigenetic events specifically related with methylation events will be considered in further research.

This work was funded by National Funds through FCT under the Project UIDB/05183/2020 (MED exploratory project ID 10\_2021).



## Functional analysis of miR399 and miR827 involved in phosphate and sugar homeostasis during the loss of the embryogenic competence in *Solanum betaceum* Cav.

Daniela Cordeiro<sup>1</sup>, Jorge Canhoto<sup>1</sup>, Sandra Correia<sup>1,2</sup>

<sup>1</sup>University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

<sup>2</sup>InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

Email: [danielacordeiro@outlook.pt](mailto:danielacordeiro@outlook.pt)

Somatic embryogenesis (SE) is a valuable tool for micropropagation and improvement of numerous economically relevant crops, and therefore there is a great interest in understanding the gene regulatory networks underlying cell reprogramming during this process. Being a maintainable system by embryogenic callus subculturing, SE in tamarillo (*Solanum betaceum* Cav.) represents a good model to study embryogenic competence acquisition, expression and maintenance<sup>1</sup>. miR399 and miR827 showed to be up-regulated in calli that lost their embryogenic potential throughout subcultures comparatively to embryogenic ones. Concomitantly, their target genes, involved in phosphate-transport responses in the cell (*PHOSPHATE2* and *PHOSPHATE TRANSPORTER 5*)<sup>2</sup>, were down-regulated in long-term calli. Since sugar signalling mediates phosphate starvation responses<sup>3</sup>, our hypothesis is that the long exposure to high sucrose concentrations during tamarillo subcultures might be responsible for the loss of the embryogenic competence. The functional validation of both miRNAs/targets is being conducted by several approaches including validation of miRNA target genes by miR-RACE, virus-based miRNAs silencing, miRNAs overexpressing, highly sensitive miRNAs FISH techniques, phosphate and sucrose quantification, changes in sucrose and/or phosphate availability in culture media. The results so far obtained corroborate the hypothesis and allow a better modulation of plant regeneration processes in very often recalcitrant plant species.

### References

<sup>1</sup>Sandra Correia, Jorge Canhoto (2018). Somatic Embryogenesis of Tamarillo (*Solanum betaceum* Cav.). In Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants, S.M. Jain and P.K. Gupta, eds. (Springer, Cham), p.171–179.

<sup>2</sup>Wei-Yi Lin, Teng-Kuei Huang, Tzyy-Jen Chiou (2013). Nitrogen Limitation Adaptation, a target of microRNA827, mediates degradation of plasma membrane-localized phosphate transporters to maintain phosphate homeostasis in Arabidopsis. *The Plant Cell*, 25, 4061–4074.

<sup>3</sup>Athikkattuvalasu Karthikeyan, Deepa Varadarajan, Ajay Jain, Michael Held, Nicholas Carpita, Kashchandra Raghothama (2007). Phosphate starvation responses are mediated by sugar signalling in Arabidopsis. *Planta*, 225, 907–918.

## Functional characterization of tRNA/rRNA methyltransferases from the SpoU family in *Arabidopsis thaliana* (L.) Heynh.

Cláudia Marinho<sup>1</sup>, Ricardo Ferraz<sup>1</sup>, Sandra Correia<sup>1,2</sup>, Jorge Canhoto<sup>1</sup>

<sup>1</sup>Center for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

<sup>2</sup>InnovPlantProtect CoLAB, Elvas, Portugal

Email: [cclaudia.cmarinho@gmail.com](mailto:cclaudia.cmarinho@gmail.com)

Somatic embryogenesis is a micropropagation technique used for plant cloning, but it is also a crucial tool to study embryo development. NEP-TC (Non-Embryogenic Protein from Tamarillo Callus, GenBank accession number JQ766254) is a protein belonging to the RNA-methyltransferases SpoU family and previous studies have suggested that it may be involved in the inhibition of somatic embryogenesis in *Solanum betaceum* Cav.<sup>1</sup>.

To understand the role of NEP-TC in somatic embryogenesis and in plant development, considering the lack of genetic resources for non-model plants, *Arabidopsis thaliana* is being used instead. Mutant lines for three genes encoding SpoU proteins, including a NEP-TC's ortholog, have been analyzed and compared with the wild-type Columbia ecotype. Regarding the plant development, germination rate, plant growth speed and root and silique development<sup>2</sup> were analyzed. The response of immature seeds to somatic embryogenesis induction<sup>3</sup> was also evaluated.

The single mutant lines showed just slight differences when compared with the wild-type line. Primary root length is smaller in mutant lines, while the number of lateral roots is higher as well as the number of total seeds per silique. Concerning somatic embryogenesis induction, mutant lines seem to slightly increase the response of explants, resulting in the formation of callus or embryos. These results need further analysis, since genetic redundancy between the SpoU proteins may affect the ability to undergo somatic embryogenesis. Thus, our goal is to obtain double and triple mutants to functionally characterize SpoU family in *Arabidopsis thaliana*.

Double mutants have already been obtained. The next step is to obtain homozygous double mutants and to perform the development and somatic embryogenesis assays in those plants.

### References

<sup>1</sup>Sandra Correia, Ana T. Alinho, Bruno Casimiro, Célia M. Miguel, Margarida Oliveira, Paula Veríssimo, Jorge Canhoto (2019). *Frontiers in Plant Science*, 10, 438.

<sup>2</sup>Douglas C. Boyes, Adel M. Zayed, Robert Ascenzi, Amy J. McCaskill, Neil E. Hoffman, Keith R. Davis, Jörn Görlach (2001). *The Plant Cell*, 13, 1499-1510.

<sup>3</sup>Miho Ikeda-Iwai, Shinobu Satoh, Hiroshi Kamada (2002). *Journal of Experimental Botany*, 53, 1575-1580.

## Fungal community of almond orchard in Alentejo region

Ana Faustino<sup>1,2</sup>, M. Margarida Oliveira<sup>3</sup>, Rosário Félix<sup>4</sup>, Liliana Marum<sup>1,2</sup>

<sup>1</sup>Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (ipBEJA), 7801-908 Beja, Portugal

<sup>2</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute CEBAL, 7801-908 Beja, Portugal

<sup>3</sup>Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Plant Functional Genomics Laboratory, 2780-157 Oeiras, Portugal

<sup>4</sup>MED (Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento) & CHANGE – Global Change and Sustainability Institute, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Apartado 94, 7002-554 Évora, Portugal

Email: [ana.faustino@cebal.pt](mailto:ana.faustino@cebal.pt)

Almond tree is a major tree nut cultivated worldwide, reaching in 2019 about 33550 tons of almond production. Portugal became the 15<sup>th</sup> almond producer worldwide and the 3<sup>rd</sup> in Europe with the introduction of new commercial orchards in Alentejo, being this region responsible for about 42% of the national production in 2020. However, these new plantations are been installed without any previous study of the edaphoclimatic conditions and some phytosanitary problems have been already detected. Fungal pathogens are associated with the decrease of the productivity of almond plantations essentially by the formation of branch and trunk canker diseases. In Alentejo, members of the genera *Fusarium* and *Alternaria* have been recently isolated from symptomatic almond trees.

The early identification of the fungal community allows the development of new strategies for future disease management minimizing future economic losses. This work pretends to identify and characterize the fungal community from an orchard in Alentejo region. Different plant materials were analyzed by molecular techniques, through DNA amplification of the internal transcribed spacer (ITS) regions of nuclear rDNA and Sanger sequencing. With this work, phytopathogenic and endophytic fungi will be identified allowing the design better management of almond orchards protection.

This work was funded by Inov-Amendo-AL (ALT20-03-0246-FEDER-000068). Authors also acknowledge FCT for Contrato—Programa to L. Marum (CEECINST/00131/2018) and FCT for UIDB/05183/2020, UIDB/04004/2020, and LA/P/0121/2020 projects.

## Genome wide association studies to wood development identifies SOBIR1, a regulator of xylem fibers differentiation

Ana Milhinhos<sup>1,2,3</sup>, Francisco Vera-Sirera<sup>4</sup>, Noel Blanco-Touriñán<sup>4</sup>, Cristina Mari-Carmona<sup>4</sup>, Àngela Carrió-Seguí<sup>4</sup>, Javier Forment<sup>4</sup>, Clément Champion<sup>3</sup>, Anna Thamm<sup>3</sup>, Cristina Urbez<sup>4</sup>, Helen Prescott<sup>3</sup>, Javier Agustí<sup>3,4</sup>

<sup>1</sup>GREEN-IT-ITQB-NOVA-Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

<sup>2</sup>FCUL, Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

<sup>3</sup>Department of Plant Sciences, University of Oxford, OX1 3RB Oxford, United Kingdom

<sup>4</sup>Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas (CSIC)-Universitat Politècnica de València (UPV), 46022 Valencia, Spain

Email: [milhinho@itqb.unl.pt](mailto:milhinho@itqb.unl.pt)

In plants, secondary growth brings about the largest portion of terrestrial biomass, in the form of wood (or xylem). Mediated by the vascular cambium, this thickening of stems and roots is an essential feature of tree growth, conferring mechanical support to the plant body and its growing structures. However, the genetic and molecular mechanisms underlying wood formation and development are still scarcely known. This is mainly because the use of classical genetics is hindered by the challenge of trees long-life cycles and the difficulty in phenotyping traits in the innermost tissues of plants. Taking advantage of the natural variation of secondary growth we performed genome-wide association studies (GWAS)-guided reverse genetics in *Arabidopsis thaliana* to discover new regulators of secondary growth. We identified *LRR-RLK EVERSHERD* (EVR), also named *SUPPRESSOR OF BIR-1 (SOBIR1)* as a regulator of xylem differentiation. We further investigated the mechanism by which SOBIR1/EVR operates in xylem fiber development and have involved the previously described master regulators BREVIPEDICELLUS (BP) and ERECTA (ER) in this process. We demonstrate that BP binds SOBIR1/EVR promoter and that *SOBIR1/EVR* expression is enhanced in *bp* mutants, suggesting a direct, negative regulation of BP over *SOBIR1/EVR* expression. We show that SOBIR1/EVR physically interacts with ER and that defects caused by the *sobir1/evr* mutation are aggravated by mutating *ER*, indicating that SOBIR1/EVR and ERECTA act together in the control of the precocious formation of xylem fiber development. We show the anatomical, genetic, and molecular evidence indicating that SOBIR1/EVR prevents the precocious differentiation of xylem fiber, a key cell type for wood development.

## Genomics and bioinformatics strategies designed to tackle *Vitis vinifera* diversity

Sara Freitas<sup>1,2,3</sup>, Antonio J. Muñoz-Pajares<sup>1,3,4</sup>, David Azevedo-Silva<sup>1,2,3</sup>, Mariana Sottomayor<sup>1,2,3</sup>, João Tereso<sup>1,3,5,6</sup>, Antero Martins<sup>7,8</sup>, Elsa Gonçalves<sup>7,8</sup>, Miguel Carneiro<sup>1,2,3</sup>, Herlander Azevedo<sup>1,2,3</sup>

<sup>1</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

<sup>3</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

<sup>4</sup>Departamento de Genética, Facultad de Ciencias, Universidad de Granada. Campus Fuentenueva, 18071, Granada, Spain

<sup>5</sup>MHNC-UP - Museum of Natural History and Science of the University of Porto - PO Herbarium, University of Porto, Praça Gomes Teixeira, 4099-002, Porto, Portugal

<sup>6</sup>Centre for Archaeology, UNIARQ, School of Arts and Humanities, University of Lisbon, Portugal

<sup>7</sup>LEAF- Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>8</sup>Portuguese Association for Grapevine Diversity-PORVID, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Email: [siofreitas@cibio.up.pt](mailto:siofreitas@cibio.up.pt); Correspondence: [hazevedo@cibio.up.pt](mailto:hazevedo@cibio.up.pt)

Grapevine (*Vitis vinifera* L.) possesses an extensive diversity, brought about by a complex domestication history over multiple historical periods. Expansion of human activity led to the creation of thousands of varieties, but the recent favoring of specific varieties/clones, and the globalization-driven exposure to pathogens, has led to extensive genetic erosion in this widely cultivated and economically significant crop. Fighting this genetic erosion whilst addressing issues of resilience to climate change, yield and other traits, requires a crucial understanding of the genetic basis of grapevine variation. Biodiversity assessments have witnessed significant advances due to the use of genomics approaches, enabled by Next Generation Sequencing. Here, NGS-driven genomics strategies have been used to tackle aspects associated with genetic diversity in grapevine. Topics include 1) a clarification of different features of its recent evolutionary history that suggest a meaningful role of the Iberian Peninsula in grapevine domestication<sup>1</sup>, and 2) the use of Pool-Seq for the quantification of genetic diversity and establishment of new phenotype-genotype associations. The genome-level analysis employ multiple genomics and bioinformatics strategies that help bridge the gap between population history, genomic variation and gene function.

This work was financed through Fundação para a Ciência e Tecnologia (FCT/MCTES) for project GrapeVision (PTDC/BIA-FBT/2389/2020) and support to H.A. (CEECIND/00399/2017/CP1423/CT0004); FCT/MCTES and POCH/NORTE2020/FSE for support to S.F. (SFRH/BD/120020/2016); FCT/MCTES and POPH-QREN/FSE for support to M.C. (CEECINST/00014/2018/CP1512/CT0002).

### References

<sup>1</sup>Freitas S et al. (2021). *Science Advances*, 7(47):eabi8584. DOI 10.1126/sciadv.abi8584

## Grapevine molecular responses to trunk diseases infection in the Alentejo region

Mariana Patanita<sup>1</sup>, Maria Doroteia Campos<sup>1</sup>, André Albuquerque<sup>1</sup>, Patrick Materatski<sup>1</sup>, Carla M. R. Varanda<sup>1</sup>, Joana A. Ribeiro<sup>1</sup>, Ricardo Ramiro<sup>2</sup>, Diana Pimentel<sup>2</sup>, Maria do Rosário Félix<sup>3</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>InnovPlantProtect Collaborative Laboratory, Estrada de Gil Vaz, Ap. 72, 7350-999 Elvas, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [mpatanita@uevora.pt](mailto:mpatanita@uevora.pt)

Grapevine trunk diseases (GTDs) have become a major concern to viticulture worldwide, causing significant economic impacts on yield and vineyard longevity. These diseases are caused by a wide range of trunk pathogens that might coexist in a plant, producing a variety of symptoms. The prevalence of GTDs is increasing and, together with the absence of effective treatments, they are currently one of the major challenges for viticulture sustainability. Identification of the regulatory components involved in grapevine protection against trunk pathogens is of high interest for disease management. This study aimed to investigate grapevine molecular responses against GTDs, following a real-time qPCR approach. Experiments were performed in three sites within the Alentejo region, and samples were collected from GTDs asymptomatic and symptomatic plants of two cultivars with different levels of susceptibility to GTDs, 'Trincadeira' and 'Alicante Bouschet'. Nine target genes previously described as involved in plant response to biotic stress were selected, namely peroxidases, transcription factors, MAPK, and pathogenesis-related proteins. Results revealed that defence-related gene expression is differently influenced by each of the factors, 'symptomatology' and 'cultivar', as well as by site. *PR1* and *TLP8* were significantly overexpressed in symptomatic plants in two sites. Similarly, *MAPKKK17* and *PR3* were overexpressed in asymptomatic ones in two sites, and *PER42* was overexpressed in all sites. *PER42* and *LOX11* were significantly overexpressed in the less susceptible cultivar, 'Trincadeira', in two sites. Interestingly, in two sites, the most susceptible cultivar, 'Alicante Bouschet', did not reveal any differentially expressed genes, which may help to better understand the reason why this cultivar is more susceptible to GTDs. Results presented here can help to explain the different susceptibilities grapevine plants have to GTDs and to identify genes that might be promising candidates for strategies involving the activation or inhibition of potential plant response regulators, offering sustainable and effective alternatives to successfully manage GTDs.

This work is funded by Portuguese National Funds through FCT/MCTES under the PhD scholarship SFRH/BD/145321/2019, attributed to Mariana Patanita, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo, and under the project UIDB/05183/2020 (MED exploratory project ID 04\_2019). It is also supported by the projects 'GAFAPROTECT' (ALT20-03-0145-FEDER-028263 | PTDC/ASP-PLA/28263/2017), and 'TOMVIRPROTECT' (ALT20-03-0145-FEDER-028266 | PTDC/ASP-PLA/28266/2017), both projects co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through the FCT, in its national component.

## Functional characterization of putative halide peroxidases in plants

Leandro Pires<sup>1,2,3\*</sup>, Tiago Lourenço<sup>1,2,3,4</sup>, Sara Freitas<sup>1,2,3</sup>, Herlander Azevedo<sup>1,2,3</sup>, Pedro Humberto Castro<sup>1,2\*\*</sup>

<sup>1</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>2</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

<sup>3</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

<sup>4</sup>BPMP, Université de Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

\*Presenting author email: [leombpires@gmail.com](mailto:leombpires@gmail.com); \*\*Corresponding author email: [pedro1berto@cibio.up.pt](mailto:pedro1berto@cibio.up.pt)

Halides play a fundamental role in plant growth, development and disease resistance. The utmost example is chloride, a plant nutrient that is a crucial factor in drought resistance, stomatal aperture and photosynthesis<sup>1</sup>. On the other hand, some halides such as iodide, are not considered nutrients but are highly beneficial for plant growth and response to environmental stress<sup>2</sup>. Although halides have several positive effects on plants, in excess, they can be highly toxic and therefore their homeostatic levels should be tightly controlled. Strangely, the knowledge about halide homeostasis in plants is very limited. Halide peroxidases (HPOs) have been identified in the brown algae *Laminaria digitata* (a halide hyperaccumulator), and are known to control halide organification in algae<sup>3</sup>. With the LdHPO's sequence, we were able to identify putative HPOs in *Arabidopsis thaliana*'s genome. In addition, the phylogenetic analysis allowed us to verify that these putative HPOs are present and conserved across the plant kingdom. The most ubiquitous member of the family will be further characterized. We analyzed the phenotype of the *Arabidopsis knockout* mutants and conclude that putative HPOs are involved in many aspects of plant development, including flowering time, shoot height and silique production. Moreover, we subjected the plants to excessive chloride treatments which clearly highlight that these putative HPOs are involved in chloride homeostasis, an important feature in response to salinity stress.

The work was mainly supported by FCT/MCTES, FEDER and COMPETE/POCI (PTDC/BAA-AGR/31122/2017, POCI-01-0145-FEDER-031122) and BIOPOLIS - European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement Number 857251).

### References

<sup>1</sup>Chen W, He ZL, Yang XE, Mishra S, Stoffella PJ (2010). *Journal of Plant Nutrition*, 33, 943-952.

<sup>2</sup>Kiferle C, Martinelli M, Salzano AM, Gonzali S, Beltrami S, Salvadori PA, Hora K, Holwerda HT, Scaloni A, Perata P (2021). *Frontiers in Plant Science*, 12.

<sup>3</sup>Colin C, Leblanc C, Wagner E, Delage L, Leize-Wagner E, van Dorsselaer A, Kloareg B, Potin P (2003). *Journal of Biological Chemistry*, 278, 23545–23552.

## Microsatellite based grapevine varietal fingerprinting throughout the wine chain

Sara Barrias<sup>1,2</sup>, Paula Martins-Lopes<sup>1,2</sup>

<sup>1</sup>Universidade de Trás-os-Montes e Alto Douro, Department of Genetics and Biotechnology, P.O. Box 1013, 5000-911 Vila Real, Portugal

<sup>2</sup>BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016

Lisboa, Portugal

Email: [sarabarrias@hotmail.com](mailto:sarabarrias@hotmail.com)

Wine varietal composition is a factor strictly related to its quality and value. The correct identification of varieties used in wine production must be assured to guarantee authenticity. PCR amplification of microsatellite markers as a DNA-based strategy for varietal identification is currently well established regarding the analysis of the vines. However, applying this methodology to must and wine samples is still a challenging approach, due to the contamination and degradation levels of the extracted DNA. Our goal for this preliminary study was to achieve successful amplification of SSR markers in gDNA extracted from different matrices. We used five varieties: Cabernet Sauvignon as international reference, two commonly used varieties in Portugal (Touriga Franca and Touriga Nacional), and two less utilized autochthonous varieties (Donzelinho Tinto and Rufete). PCR reactions were performed using nine SSR markers recommended by OIV<sup>1,2</sup> and gDNA samples extracted from leaf, must and monovarietal wines. Amplicons were separated by capillary electrophoresis followed by allele scoring. All markers were successfully amplified in leaf gDNA samples. Amplification in must gDNA samples was also successful for all varieties and most markers, excluding VVMD25 and VVMD32, both producing larger amplicons. Different markers were amplified in wine gDNA from all varieties, and Cabernet Sauvignon showed the lowest success rate of amplification, due to presenting the lowest concentration and the highest contamination levels, which hampers PCR reactions. A comparison between sample types for individual SSR loci will determine the feasibility of this approach for the establishment of an authenticity plan in must and wine samples, which can be applied from grape winery reception to commercialized wine in a bottle.

Fundação para a Ciência e a Tecnologia for the BioISI project (reference Nr. UID/MULTI/04046/2021) and the Ph.D Grant SFRH/BD/146346/2019 to S.B.

### References

<sup>1</sup>OIV. 2<sup>nd</sup> edition of the OIV Descriptor list for grape varieties and Vitis species. Paris, 2007.

<sup>2</sup>OIV General Assembly. OIV Protocol for Identification of Varieties. Geneva, Switzerland, The General Director of OIV - Pau Roca Blasco, 2019.



## Morphological and genetic diversity in *Cynara cardunculus* L.

Jacqueline Santos<sup>1,2</sup>, Maria Castro<sup>1</sup>, Daniela Rosa<sup>1</sup>, Ana F. Paulino<sup>1,4</sup>, Liliana Marum<sup>1,2</sup>, Maria F. Duarte<sup>1,2</sup>, Anabela Belo<sup>3</sup>, Carla P. Cruz<sup>3</sup>

<sup>1</sup>Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, Beja, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute; Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora

<sup>4</sup>Computational Biology and Population Genomics Group, Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

Email: [jacqueline.oliveira@cebal.pt](mailto:jacqueline.oliveira@cebal.pt)

During the last three decades, *Cynara cardunculus* L (cardoon) has intensively been researched and recently became a commercial crop for its multifaceted industrial applications, with pharmacological and nutritional properties<sup>1,2</sup>. For different purposes, Flowers, leaves and total biomass are harvested either in the wild or in cultivated populations. Due to its biological potential, it's critical to gain knowledge about its diversity, especially in the management of in situ wild populations aiming to enhance the abundance of plants with desirable phenotypes<sup>3,4</sup>.

The present study aimed to evaluate the morphological and genetic variation of *Cynara cardunculus* L. We compare the diversity through several morphological descriptors (plant height, number of stems, number of secondary ramifications, total of inflorescence, length of primary ramification, leaf width at 1<sup>st</sup> floral branch, stalk maximum and minimum diameter on top and base, leaf length, leaf fresh and leaf dry weight) and SSR markers of 35 individuals of *C. cardunculus* from 5 wild populations (Jurumenha, Monte da chaminé, Herdade do Peral, Herdade da Revilheira e Herdade de São Romão) of the Portuguese Alentejo region. We analysed morphological diversity indices (IMD, based on Simpson's index). Morphological diversity (IMD) was similar among the population ranging from 0.298 to 0.318. However, Herdade da Revilheira and Herdade de São Romão populations showed averaged higher genetic variation than Monte da Chaminé, Herdade do Peral and Juromenha.

These results illustrate that wild *Cynara cardunculus* populations are important reservoirs of genotype variation and thus are crucial for the genetic diversity safeguard. These populations may play a fundamental role in designing strategies for conservation and will provide valuable information for future management of *C. cardunculus* germplasm.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), Ph.D. grant to A. Paulino (SFRH/BD/145383/2019), and D. Rosa (SFRH/BD/143845/2019), Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and Project LA/P/0121/2020 to CHANGE - Global Change and Sustainability Institute.

### References

- <sup>1</sup>Ramos PAB, Guerra AR, Guerreiro O, et al. Lipophilic extracts of *Cynara cardunculus* L. var. altilis (DC): A source of valuable bioactive terpenic compounds (2013). *Journal of Agricultural and Food Chemistry*. 61(35):8420-8429.
- <sup>2</sup>Petropoulos SA, Pereira C, Tzortzakis N, Barros L, Ferreira ICFR. Nutritional value and bioactive compounds characterisation of plant parts from *Cynara cardunculus* L. (Asteraceae) cultivated in central Greece (2018). *Frontiers in Plant Science*. Published online.
- <sup>3</sup>Gominho J, Curt MD, Lourenço A, Fernández J, Pereira H. *Cynara cardunculus* L. as a biomass and multi-purpose crop: A review of 30 years of research (2018). *Biomass and Bioenergy*. Published online. doi:10.1016/j.biombioe.2018.01.001.
- <sup>4</sup>Castro MM, Rosa D, Ferro AM, et al. Genetic diversity and population structure of *Cynara cardunculus* L. in southern Portugal (2021). *PLOS ONE*. 16(6):e0252792. doi:10.1371/JOURNAL.PONE.0252792.

## Non-embryogenic cells transformation and identification of NEP-TC RNA targets in tamarillo

Ricardo Ferraz<sup>1,2</sup>, Bruno Casimiro<sup>1</sup>, Daniela Cordeiro<sup>1</sup>, Sandra Correia<sup>1,3</sup>, Sílvia Coimbra<sup>2,4</sup>, Jorge Canhoto<sup>1</sup>

<sup>1</sup>Center for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

<sup>2</sup>LAQV Requimte, Sustainable Chemistry, University of Porto, Porto, Portugal

<sup>3</sup>InnovPlantProtect CoLAB, Elvas, Portugal

<sup>4</sup>Faculty of Sciences of the University of Porto, Porto, Portugal

Email: [rikayferr@hotmail.com](mailto:rikayferr@hotmail.com)

Although seeds are the plant's propagation unit, sexual reproduction does not ensure genetic uniformity. Thus, asexual methods of propagation need to be employed for the propagation of hybrids or preferred genotypes and somatic embryogenesis (SE) has proven to be a valuable tool for plant breeding and to understand embryo development. Tamarillo, a solanaceous tree that produces fruits of high nutritional and economic value, presents characteristics that make it an appropriate plant to study SE. The NEP-TC (Non-Embryogenic Protein from Tamarillo Callus, GenBank accession number JQ766254) enzyme is a member of the SpoU methyltransferase family that is involved in SE inhibition<sup>1</sup>. However, its biological mechanism of action is still unknown. To determine NEP-TC targets, a cross-linking and analysis of cDNA (CRAC)<sup>2</sup> method is being performed. This method will submit tamarillo non-embryogenic callus (NEC) cells to an agent that will induce cross-links in the complex that involves the NEP-TC and its RNA targets, which will be subsequently purified. These RNA targets will then be reverse transcribed, being the respective cDNA amplified and sequenced. Lastly, the genes that correspond to these targets will be identified by comparison of these sequences to conserved coding regions of other *Solanum* species with their genome sequenced. NEP-TC coding sequence was already recombined with a histidine tail sequence and cloned into an entry vector that will be recombined with a destination vector containing the 35S promoter, that will induce the constitutive expression of the recombined NEP-TC gene in tamarillo NEC cells prior to the cross-linking. To enable the transformation of tamarillo NEC cells with the recombinant NEP-TC gene, an *Agrobacterium* mediated transformation protocol was optimised using the p35SGUSINT vector. The success of the transformation protocol was confirmed by observation of  $\beta$ -glucuronidase (GUS) staining in transformed NEC cells and GUS gene amplification in genomic DNA samples from these cells.

### References

<sup>1</sup>Sandra Correia, Ana T. Alinho, Bruno Casimiro, Célia M. Miguel, Margarida Oliveira, Paula Veríssimo, Jorge Canhoto (2019). *Frontiers in Plant Science*, 10, 438.

<sup>2</sup>Sara Haag, Jens Kretschmer, Katherine E. Sloan, Markus T. Bohnsack (2017). *RNA Methylation*, 1562, 269-281.

## Optimization of enzymatic production in tamarillo cell suspensions cultures

Bruno Casimiro<sup>1</sup>, Mariana Correia<sup>1</sup>, Jorge Canhoto<sup>1</sup>, Paula Veríssimo<sup>2</sup>, Sandra Correia<sup>1,3</sup>

<sup>1</sup>University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

<sup>2</sup>Centre for Neuroscience and Cell Biology, University of Coimbra, Faculty of Medicine, Polo I, 1st floor, 3004-504, Coimbra, Portugal

<sup>3</sup>InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

Over the last decades, plants have been efficiently used as platforms for the rapid and scalable production of valuable metabolites, with the intrinsic safety of food crops. Plant-based production of recombinant proteins is now a rapidly developing segment of the bioeconomy.

Within the available Molecular Farming platforms, plant cell suspensions (PCS) are sustainable and efficient systems for producing high-quality molecules with rapid growth, protein consistency using controlled bioreactors under contained environments, and fewer issues with pathogen contamination involving viral or bacterial toxins. Due to simple and cheaper growth conditions, PCS cultures offer a low-cost alternative to mammalian production systems.

Tamarillo (*Solanum betaceum* Cav.) is a particularly interesting system used as an experimental model for plant development and biotechnological processes, in which our team has vast experience, including working on the molecular analysis of stress-induced regeneration processes.

In this work, tamarillo *calli* lines were grown efficiently in PCS cultures, and their protein patterns were analyzed, showing the production of a wide array of hydrolytic enzymes and low molecular weight peptides (>20 kDa). By using different biotic elicitors (e.g., chitosan, yeast extract), enzyme production was enhanced, particularly for chitosan (0.1g/l) elicited extracts showing higher glycosidase, alkaline phosphatase, and protease activities. Further proteomic analysis is being conducted to identify differentially expressed proteins between treatments and the effectiveness of the obtained extracts assayed on bioactivity tests. Furthermore, we are evaluating the effect of deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA), which has been reported as an enhancer for protein production in PCS, with promising results.

This work was supported by the Foundation for Science and Technology (Portugal) through a doctoral research fellowship awarded to Bruno Casimiro (SFRH/BD/146485/2019) and the P2020|COMPETE grant number PTDC/BAA-AGR/32265/2017, BP4BP – Tamarillo breeding: better plants for better products, and the University of Coimbra / Santander Seed Projects Award 2021.

**Overexpression of *QsMYB1* gene from *Quercus suber* induces suberin alteration in transgenic potato plants**

Ana Faustino<sup>1,2</sup>, Rita Pires<sup>1</sup>, Tiago Capote<sup>1</sup>, Joana Serôdio<sup>1</sup>, Sérgio João<sup>1</sup>, Leonor Morais-Cecílio<sup>3</sup>, José Graça<sup>4</sup>,  
Liliana Marum<sup>1,2</sup>

<sup>1</sup>Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

<sup>2</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute CEBAL, 7801-908 Beja, Portugal

<sup>3</sup>Linking Landscape, Environment, Agriculture and Food (LEAF) Instituto Superior de Agronomia, University of Lisbon, Lisboa, Portugal

<sup>4</sup>Forest Research Center (CEF) Instituto Superior de Agronomia, University of Lisbon, Lisboa, Portugal.

Email: [ana.faustino@cebal.pt](mailto:ana.faustino@cebal.pt)

Periderm is a protective tissue present in plants with secondary growth, composed of phellem, phellogen, and phelloderm. These different cell types are formed from the phellogen, a layer of meristematic cells with high division capacity, by a process that includes cell expansion, suberization of the cell walls, deposition of waxes, and cell death. The main component of periderm is suberin, a complex glycerol-based polymer consisting of an aliphatic polyester linked with phenolic compounds and embedded waxes. *QsMYB1* gene is a member of the R2R3-MYB transcription factor family involved in the formation and differentiation of periderm by targeting genes associated with the biosynthesis pathways of suberin and lignin in cork oak.

A reverse genetic approach was developed in *Solanum tuberosum* for functionally characterizing the *QsMYB1* gene. Potato periderms of plants overexpressing *QsMYB1* and from wild type plants have been analyzed by GC-MS. The results showed that the overexpression of *QsMYB1* induced changes in the composition of suberin with a decrease in glycerol content and alterations in the composition of  $\alpha,\omega$ -diacids and  $\omega$ -hydroxiacids, the two principal fatty acids families of suberin. These results reinforce the importance of this transcriptional factor in the biosynthesis pathway of suberin.

This Work was supported by Alentejo2020, through FEDER under Lentidev-ALT20-03-0145-FEDER-000020 project and national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit. Authors also acknowledge FCT for Contrato—Programa to L.Marum (CEECINST/00131/2018) and FCT for UIDB/05183/2020, and LA/P/0121/2020 projects.

## Phenotypic and molecular characterization of selected strawberry tree (*Arbutus unedo* L.) clones

Ricardo Pereira<sup>1,2</sup>, Mayara da Silva<sup>2</sup>, Carolina Dias<sup>2</sup>, Carlos Godinho<sup>3</sup>, Alcinda Neves, José Leitão<sup>2</sup>

<sup>1</sup>Corte Velada Investimentos, Odiáxere, Portugal

<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute. Laboratory of Genomics and Genetic Improvement, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139, Portugal

<sup>3</sup>DGAV – Direção-Geral de Alimentação e Veterinária, Tapada da Ajuda 1349-017 Lisboa.

Email: [ricper1990@gmail.com](mailto:ricper1990@gmail.com) ; [jleitao@ualg.pt](mailto:jleitao@ualg.pt)

Strawberry tree (*Arbutus unedo* L.) is an evergreen small fruit tree belonging to the Ericaceae family, well adapted to the environmental conditions of the Mediterranean basin, where it grows as a main species of the local flora. In Portugal, particularly in the Algarve region, this species is of relative economic importance since the produced berries are traditionally used to produce jams and the liquor “aguardente de medronho”. The present alteration of the traditional production by small farmers to cultivation in larger orchards by modern companies requires the replacement of the commonly used highly heterogenous seed propagated plants by homogeneous, selected, or improved, vegetatively propagated clones. To balance the adverse impact, of this new production trend, in the genetic variability of the species, in collaboration with the University of Algarve, the enterprise Corte Velada Investimentos has been collecting, selecting and vegetatively propagating genotypes with different characteristics, establishing a publicly accessed germplasm field collection. Here, we report the preliminary analysis of the phenotypic and genetic variability of 30 selected clones. The phenotypic analysis was based on the crop descriptor newly developed by the DGAV: “Guidelines for the conduct of tests for distinctness, uniformity and stability of *Arbutus Unedo* L.”. The genomic DNA of the same genotypes was analyzed by RAPD and ISSR markers, using five primers of each kind of techniques, which amplified 64 clearly scorable markers that allowed to assess the genetic variability and the genetic similarity relationships among this sample of selected accessions.

This work was funded by the project: Operação 7.8.4 Conservação e melhoramento de recursos genéticos vegetais, PDR2020-7.8.4-FEADER-042697.

## Phylogenetic relationships in *Vigna germplasm* - contribution for future breeding programs

Carvalho Márcia<sup>1,2</sup>, Carnide Valdemar<sup>1,2</sup>, Rosa Eduardo<sup>1,2</sup>, Castro Isaura<sup>1,2</sup>

<sup>1</sup>Centre for Research and Technology of Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal,

<sup>2</sup>Institute for Innovation, Capacity Building and Sustainability of Agri-food Production (Inov4Agro), University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal

Email: [marciac@utad.pt](mailto:marciac@utad.pt)

The genus *Vigna* belongs to the family Leguminosae and includes more than 150 cultivated and wild species distributed throughout the world, presenting a high economic and social importance, particularly in the developing countries. This genus has great variability and contains some of the most important cultivated grain legumes species, as cowpea (*Vigna unguiculata* L. Walp.) and mung bean (*Vigna radiata* L. Walp.).

Agricultural production worldwide is threatened by the effects of climate change. Underused cultivated germplasm and wild relative crops are thought as reservoir of prominent genetic resources for abiotic stresses tolerance, such as high temperatures, drought, low soil fertility, and will be crucial to obtain new varieties. Nevertheless, the information available on genetic diversity in the genus *Vigna* is insufficient. Microsatellites (SSRs) are an excellent marker system for accessions DNA fingerprinting and genetic diversity studies.

To evaluate the genetic diversity and phylogenetic relationships in the genus *Vigna*, a set of six SSR loci was analysed in 40 accessions, including four different *Vigna* species and six subspecies of *Vigna unguiculata*. Five loci amplified were polymorphic and a total of 27 alleles was detected, ranging from one (CLM0117) to seven (CLM0260) alleles per locus. Thirty-three genotypes were detected, of which 13 were considered unique. The dendrogram obtained revealed a coefficient of similarity varying from 32 to 100%. This study allowed to assess the diversity within the genus *Vigna* and *Vigna unguiculata* subspecies and to analyse the phylogenetic relationships between them. A slight common genetic background between the material under study was observed, being important to complement the analysis with a greater number of SSR loci and accessions to provide full assessment of genetic diversity to be to be exploited by breeders in the future.

This study was supported by CITAB project funding from National Funds by FCT – Portuguese Foundation for Science and Technology [grant number UIDB/04033/2020].

## Quercus suber tissues laser microdissection followed by RNA-Seq analysis

Rita Costa Pires<sup>1</sup>, Ana Usié<sup>1,2</sup>, Ana Ferro<sup>1,2,3</sup>, Tiago Capote<sup>1,2,3</sup>, Liliana Marum<sup>1,2</sup>

<sup>1</sup>Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal

<sup>3</sup>Current Address: Center for Genomics and Systems Biology, New York University Abu Dhabi, NYUAD Campus, 129188 Abu Dhabi, United Arab Emirates

Email: [rita.pires@cebal.pt](mailto:rita.pires@cebal.pt) ; [liliana.marum@cebal.pt](mailto:liliana.marum@cebal.pt)

The cork oak tree (*Quercus suber* L.) has the capability to regenerate new layers of the outer bark leading to the production of cork repeatedly, every nine years after the first extraction. The cork from the cork oak tree offers exceptional physical and chemical properties for industrial applications.

Laser Microdissection, a very precise technique for isolating cells or tissue of interest, followed by RNA sequencing allows a comprehensive transcriptome characterization of selected tissues.

In order to elucidate the molecular and biochemical events occurring in the development and differentiation processes of cork, we have developed an efficient protocol for RNA isolation from different *Q. suber* tissues isolated by laser microdissection, suitable for sequencing and further transcriptomic analysis. The extracted RNA was converted to cDNA, and then subjected to stranded paired-end sequencing using the Illumina HiSeq 2500 platform.

Differential expression analysis is ongoing to identify, accurately, distinct gene expression patterns between tissues, which would provide new insights of how cells-specific gene expression can modulate the activation of different biological pathways between the tissues studied.

Work was supported by Alentejo2020, through FEDER under Lentidev-ALT20-03-0145-FEDER-000020 project. Authors also acknowledge FCT for Contrato—Programa to L.Marum (CEECINST/00131/2018) and FCT for UIDB/05183/2020, and LA/P/0121/2020 projects.

## Studies on genetic transformation of *Olea europaea* L. mediated by *Agrobacterium tumefaciens*

Rita Pires<sup>1\*</sup>, Diogo Marques<sup>2\*</sup>, Augusto Peixe<sup>3</sup>, Hélia Cardoso<sup>1</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Formação e Investigação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal.

<sup>2</sup> Escola de Ciência e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>3</sup>MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

\* These authors contributed equally to this work.

Email: [rnpires@uevora.pt](mailto:rnpires@uevora.pt), [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

Genetic engineering can be used to speed up the development of elite olive cultivars by the rapid introduction of interesting agronomical traits in selected genotypes. Few genetic transformation protocols have been described in olives and none was based on Portuguese cultivars. Aiming to establish a procedure to assist molecular breeding of Portuguese elite cultivars a genetic transformation protocol mediated by *Agrobacterium tumefaciens* was developed using the cv. 'Galega vulgar', which is recognized by the production of high-quality olive oils. Somatic embryogenic *calli*, previously achieved by *in vitro* culture of radicle and cotyledons taken from mature zygotic embryos and further maintained through cyclic embryogenesis (for details see Pires et al. 2020<sup>1</sup>), were used as explant tissue for inoculation with *A. tumefaciens* strain AGL1 (gently provided by Ricardo Fernandez). The binary vector pk7WG2, carrying the  $\beta$ -glucuronidase (GUS) gene (used to determine the frequencies of transient gene expression) and the kanamycin (NPTII) gene (used as a selectable marker for stable integration) was used. Embryogenic *calli* were inoculated with a suspension culture of *A. tumefaciens* (0.5 OD<sub>600</sub> nm) and maintained at 25°C under dark conditions for 2 days. After the co-culture period the bacteria was removed by washing plant tissue with liquid ECO medium containing 250 mg/l cefotaxime. *Calli* were further inoculated in solid ECO medium supplemented with 250 mg/l cefotaxime. The analysis of the transient GUS gene expression was performed by histochemical assays two days after co-culture period. The material was incubated in 250  $\mu$ l of 5 bromo-3-chloro-3-indolyl D-glucuronic acid solution (X-Gluc) (400g/ml) overnight at 37 °C in the dark. The workflow developed will be presented.

### References

<sup>1</sup>Pires R, Cardoso H, Ribeiro A, Peixe A, Cordeiro A (2020). Somatic Embryogenesis from Mature Embryos of *Olea europaea* L. cv. 'Galega Vulgar' and Long-Term Management of Calli Morphogenic Capacity. *Plants*, 9, 758.



## The Date Palm Genome Diversity Project at NYU Abu Dhabi

Ana Margarida Ferro<sup>1</sup>, Tiago Capote<sup>1</sup>, Sylvie Ferrand<sup>1</sup>, Jonathan Flowers<sup>1,2</sup>, Muriel Gros-Balthazard<sup>1,3</sup>, Khaled Hazzouri<sup>1,4</sup>, Michael Purugganan<sup>1,2</sup>

<sup>1</sup>Center for Genomics and Systems Biology, New York University Abu Dhabi, 129188 Abu Dhabi, United Arab Emirates

<sup>2</sup>Center for Genomics and Systems Biology, New York University, New York, USA

<sup>3</sup>UMR Diversité Adaptation et Développement des plantes unit, Institut de Recherche pour le Développement, Université de Montpellier, 34394 Montpellier, France

<sup>4</sup>Khalifa Center for Genetic Engineering and Biotechnology, United Arab Emirates University, Al-Ain, Abu Dhabi, UAE

Email: [ana.ferro@nyu.edu](mailto:ana.ferro@nyu.edu)

Date palms are one of the earliest domesticated trees in the world. Although, culturally and economically important in the Middle East and North Africa (MENA), the biology of date palms remains unclear. However, in the last years, there have been major advances in genomic studies of this species. The Date Palm Genome Diversity research group of the Center for Genomics and Systems Biology (CGSB) at New York University Abu Dhabi (NYUAD) has sequenced the genomes of a large number of samples of date palms and its wild relatives from the MENA region. This information allows the study of the genomic diversity of date palms to understand its domestication and evolution, and to identify genes that may help in breeding and trait improvement of the species. Recent studies at NYUAD on date palm genomes have enabled new discoveries for the identification of genes underlying major traits, such as sex, fruit color, and sugar composition. In the meeting, the team will present the main achievements and discoveries about date palm genomes and will highlight their importance for agriculture and food security.

## Transcriptome profiling of indole-3-butyric acid-induced adventitious root formation in *Olea europaea* L. – a focus in the induction phase

Hélia Cardoso<sup>1</sup>, Catarina Campos<sup>1</sup>, Conceição Egas<sup>2,3</sup>, Augusto Peixe<sup>4</sup>

<sup>1</sup> MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Formação e Investigação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>Biocant- BiocantPark, Núcleo 04 Lote 8, 3060-197 Cantanhede, Portugal

<sup>3</sup>Center for Neuroscience and Cell Biology, Rua Larga - Faculdade de Medicina, 1ºandar - POLO I, University of Coimbra, 3004-504 Coimbra, Portugal

<sup>4</sup>MED - Mediterranean Institute for Agriculture, Environment and Development, Department of Plant Science, School of Science and Technology, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

Adventitious root (AR) formation is a limiting step in vegetative propagation of some agronomical interesting woody-plant species. Olive (*Olea europaea* subsp. *europaea* L.) is one of the most important fruit trees, comprising several cultivars with reduced ability for AR formation. To have a broader view into the molecular mechanisms underlying AR formation in olive, the transcriptomic changes that occur during the induction phase were analyzed. *In vitro* growing explants of cv. ‘Galega vulgar’ were used to establish the rooting assays. The indole-3-butyric acid (IBA) was used as the root induction factor. Samples were collected at 6, 24 and 72 hours post-induction (hpi) from IBA-treated and control microshoots. The dynamics of mRNA and microRNA (miRNA) transcriptomes were analysed between treatments and throughout time using the *edgeR* and *WGCNA* packages implemented in R. Several genes responsive to IBA were identified in all time points, including auxin-related genes, ethylene-responsive transcription factors, polygalacturonases and *LOB* genes, amongst others. Gene and miRNA clustering through *WGCNA* package identified modules co-related to IBA treatment and to hpi. Particularly, the ‘black’ module, positively related to IBA and hpi, showed as hub genes several *SIEVE ELEMENT OCCLUSION B-like* genes, which encode proteins related to phloem development, the gene encoding for Protein IQ-DOMAIN 14, which is related to calmodulin-binding and secondary cell wall biogenesis, and an ABC class G transporter. Gene Ontology (GO) analysis showed that the terms “response to stress”, “regulation of cellular process” and “transport” were up-regulated in this module. GO analysis of predicted target genes of a miRNA module related to hpi further showed an enrichment in terms such as “sucrose catabolic process”, “cellular protein catabolic process” and “auxin-activated signaling pathway”. These results confirmed that AR induction in olive is a complex process that depends on multiple signaling and metabolic pathways.

Authors would thank to Virginia Sobral and Isabel Velada by the help on the establishment of the *in vitro* trials. This work is supported by National Funds through FCT under the Project UID/AGR/00115/2019 and co-funded by FEDER through ALENTEJO 2020 under ALT20-03-0145-FEDER-000014.

## Unequivocal identification of *Arbutus unedo* L. clones by species specific SSR and SNP markers.

Ricardo Pereira<sup>1,2</sup>, Isabela Vera<sup>2,3</sup>, Mayara da Silva<sup>2</sup>, João Reis<sup>2</sup>, José Leitão<sup>2</sup>

<sup>1</sup>Corte Velada Investimentos, Odiáxere, Portugal

<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute. Laboratory of Genomics and Genetic Improvement, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139, Portugal

<sup>3</sup>UNEMAT – Universidade do Estado de Mato Grosso, Tangará da Serra, Mato Grosso, Brasil

Email: [ricper1990@gmail.com](mailto:ricper1990@gmail.com) ; [jleitao@ualg.pt](mailto:jleitao@ualg.pt)

Strawberry tree (*Arbutus unedo* L.), a small fruit tree of the Ericaceae family, is a main species of the natural flora and an ecologically relevant species to the Mediterranean ecosystems. Although edible, the produced berries are mostly used for production of jams and, in the Algarve region, for the production of the liquor “aguardente de medronho”. The increasing interest of modern companies in the cultivation of *A. unedo* in large scale, implies the selection and massive vegetative propagation of elite genotypes with superior characteristics, able to increase the ecological and economic viability of this fruit crop. Aiming at the conservation of the natural plant resources and use of the genetic variability for direct production and further plant breeding purposes, a germplasm field collection of this species was established by the company Corte Velada Investimentos. From a first *A. unedo* genome assembly that we have recently published (ncbi), we mined out 500 SSR (microsatellite) loci and 500 SNP (single nucleotide polymorphism). Twenty-five SSR loci and 10 SNPs loci were selected for further evaluation. The variants of all 10 SNP loci were discriminated by the same restriction enzyme: *TaqI*. After a first analysis, 4 SSR and 3 SNPs loci were retained for the genetic analyses of a set of 20 selected clones. As expected, the SSR loci were hiper-polymorphic, allowing the identification of 90 different alleles. The 12 different alleles of the 3 SNP loci were revealed by their analysis as CAPS (cleaved amplified polymorphic sequences) using the restriction enzyme *TaqI*. These markers allowed the establishment of clear, individual, and unequivocal fingerprint of the 20 selected clones.

This work was funded by the project: Operação 7.8.4 Conservação e melhoramento de recursos genéticos vegetais, PDR2020-7.8.4-FEADER-042697.

## Unequivocal identification of *Diplotaxis (L.) DC tenuifolia* accessions by species specific SSR and SNP markers

João Reis<sup>1</sup>, Ricardo Pereira<sup>2</sup>, Paula Coelho<sup>3</sup>, José Leitão<sup>1</sup>

<sup>1</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>2</sup>Corte Velada Investimentos, Odiáxere, Portugal

<sup>3</sup>INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, Av. da República, 2784-505 Oeiras, Portugal

Email: [jleitao@ualg.pt](mailto:jleitao@ualg.pt), [paula.coelho@iniav.pt](mailto:paula.coelho@iniav.pt), [a61179@ualg.pt](mailto:a61179@ualg.pt)

Wild rocket (*Diplotaxis tenuifolia* (L.) DC) is currently commercialized as baby leaf salad in individual packages or as a major component of salad leaf mixtures. The high content of glucosinolates and the release of volatile isothiocyanates, provides this crop with a spicy and bitter taste, and a strong aroma, that determines the growing consumer preference over the “cultivated” rocket (*Eruca sativa* L.). A germplasm collection of wild rocket, that includes some accessions of *Eruca* spp., is being established at the INIAV (Oeiras), which requires unequivocal identification of the gathered accessions. For this purpose, the most appropriated are the DNA markers technologies, particularly, genome specific Single Sequence Repeats (SSR) and SNPs (Single Nucleotide Polymorphisms) markers. Here, we describe the first attempt of unequivocal identification of 29 wild rocket and 1 “cultivated” rocket, germplasm accessions. Twenty out of 500 SSR *loci* and 20 out of 500 SNP *loci*, mined out from the first genome assembly of *D. tenuifolia* ([ncbi.nlm.nih.gov/assembly/GCA\\_014822095.1](https://ncbi.nlm.nih.gov/assembly/GCA_014822095.1)), were selected and tested as SSR and SNP markers. A total of 5 SSR and 9 SNP markers were retained for further accession identification. The amplified 80 SSR markers were analyzed by capillary gel electrophoresis and the amplified SNP markers were analyzed as 36 TaqI generated CAPS (cleaved amplified polymorphic sequence) markers, revealed by agarose gel electrophoresis. As expected, small groups of accessions displayed identical DNA fingerprints (100% similarity), confirming those accessions as the same commercial variety, although provided by different producers. The *Eruca* spp. accession exhibited very different and unique molecular patterns, which resulted in the lowest (0,118) genetic similarity towards the remaining accessions.

This work was funded by the project: PTDC/ASP-PLA/28963/2017- REMIRucula – Resistance characterization to downy mildew in wild rocket crop.

## Unravelling the role of DNA methylation in cork formation

Rafael Prazeres<sup>a</sup>, Ana Milhinhos<sup>ab</sup>, Pedro Barros<sup>c</sup>, José Graça<sup>d</sup>, Leonor Morais-Cecílio<sup>e</sup>, Célia M. Miguel<sup>a</sup> and Vera Inácio<sup>a</sup>

<sup>a</sup>BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

<sup>b</sup>Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

<sup>c</sup>Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), GPlantS, Av. da República, 2780-157 Oeiras, Portugal

<sup>d</sup>Forest Research Center (CEF), Institute of Agronomy, University of Lisbon, 1349-017 Lisbon, Portugal

<sup>e</sup>Linking Landscape, Environment, Agriculture and Food (LEAF), Institute of Agronomy, University of Lisbon, 1349-017 Lisbon, Portugal

Email: [rafaelprazer9@gmail.com](mailto:rafaelprazer9@gmail.com)

The periderm protects plants from adverse conditions and is made up of cork cells produced by phellogen. The cork oak phellogen (*Quercus suber*) produces an extraordinarily thick periderm that can be sustainably harvested at 9-years interval. The cork mass is interrupted by lenticular channels, the so-called cork porosity, which, at high levels, strongly depreciates the quality of cork.

The factors that determine the quality of cork are unknown, however, recent studies have shown an association between epigenetics and phenotypes directly linked to phellogen activity: (I) variations in DNA methylation are associated with cork contrasting phenotypes directly linked to phellogen activity<sup>1</sup>; (II) opposite global DNA methylation levels are observed in corks with contrasting phenotypes (higher/lower porosity)<sup>2</sup>; (III) DNA methyltransferases genes expression is correlated with cork quality traits<sup>3</sup>; and (IV) de novo DNA methyltransferase gene expresses at high levels during cork formation<sup>2</sup>. The hypothesis of a causal relationship between DNA methylation and cork phenotypic variability will be tested using potato tuber periderm as a model, due to constraints in using cork oak for functional studies via genetically modified plants. To generate loss-of-function mutants of DNA methyltransferase genes specifically in the phellogen, we are using an inducible genome editing system (IGE) that combines CRISPR/Cas9 with a cell-type-specific estrogen inducible system<sup>4</sup>. So far, the allelic composition of *MET1*, *DRM1*, *DRM2*, *CMT2*, and *CMT3* potato genes was determined to design two specific guide RNA spacers targeting the four alleles. Afterwards, constructs targeting *DRM1*, *CMT2*, and *CMT3* genes were generated by producing entry vectors by Golden Gate cloning and then recombined through Multisite Gateway LR reaction into final IGE constructs. Potato leaves and internodes were infected with *Agrobacterium tumefaciens* cells carrying the *CMT3*-targeting IGE construct. In the future, *CMT3* editing will be induced in microtubers from transformed plant lines and mutants will be genotyped and phenotyped.

### References

- <sup>1</sup>Inácio V, Barros PM, Costa A, et al (2017). *PLoS One*, 12(1), 1-18.
- <sup>2</sup>Inácio V, Martins MT, Graça J, Morais-Cecílio L (2018). *Front Plant Sci*, 1-18
- <sup>3</sup>Ramos M, Rocheta M, Carvalho L, Inácio V, Graça J, Morais-Cecílio L. (2013). *Tree Genet Genomes*, 9(6), 1481-1492.
- <sup>4</sup>Wang X, Ye L, Lyu M, Ursache R, Löytynoja A, Mähönen AP (2020). *Nat Plants*, 6(7), 66-772.

## Patterns of Suberization in *Arabidopsis thaliana* root endodermis in loss-of-function mutants of 'cork-related' candidate genes

Manuel Cavaco<sup>1</sup>, Susana Lopes<sup>1,2</sup>, Ana Milhinhos<sup>1,2</sup>, Célia Miguel<sup>1</sup>

<sup>1</sup>FCUL, Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

<sup>2</sup>GREEN-IT-ITQB-NOVA-Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Email: [fc55827@alunos.fc.ul.pt](mailto:fc55827@alunos.fc.ul.pt)

Suberin is a complex biopolymer deposited in the bark of trees. An extreme example of suberin deposition can be found in the bark (cork) of cork oak trees. In the *Arabidopsis thaliana* root tissues suberization also occurs, protecting the root from biotic/abiotic stresses. There are mainly two suberised tissues in the *Arabidopsis thaliana* roots: (1) the endodermis, that differentiates inwards to the cortex, close to the RAM, and progresses along the root axis giving rise to (2) the periderm, a protective outer tissue more prominent at the root-hypocotyl. Recently, a transcriptomic study performed at our lab revealed several promising candidate genes to be exclusively involved in cork differentiation in cork oak<sup>1</sup>. By making use of a simpler model system<sup>2</sup>, we analysed the loss of function mutants for the homolog candidate genes as to their periderm development phenotypes at the root-hypocotyl border in *Arabidopsis*. We found that mutants for the candidates: *WUSCHEL-RELATED HOMEODOMAIN 9 (WOX9)*, a homeobox gene required for SAM growth; *AINTEGUMENTA (ANT)*, a member of the *APETALA2*-like family of transcription factor genes; and genes that code for the MYB DOMAIN PROTEIN 36 (*MYB36*) showed significant changes in the width of periderm tissues when compared to the wild type. To investigate if the changes observed in the mutant's periderm were caused by alterations in suberin deposition patterns during primary growth at initial stages of endodermis development, we are analysing the root patterns of suberization using the fluorescent FluoroJ Yellow stain to 7-day old wild-type and mutant seedlings<sup>3</sup>. By examining the suberization patterns of the root endodermis, our preliminary results reveal that *wox9*, *ant-9* and *myb36* shows delayed suberization, when compared to the wild type. We are currently artificially-inducing suberization in the mutants using hormones<sup>4</sup> to further explore the possible regulatory roles for these genes and their positioning in potential existing regulatory pathways.

### References

<sup>1</sup>Lopes, S. T., Sobral, D., Costa, B., ... & Miguel, C. M. (2020) *Tree Physiology*, 40, 129-141.

<sup>2</sup>Wunderling, A., Ripper, D., Barra-Jimenez, A., ... & Ragni, L. (2018) *New Phytologist*, 219, 216-229.

<sup>3</sup>Barberon, M., Vermeer, J. E. M., De Bellis, ... & Geldner, N. (2016) *Cell*, 164, 447-459.

<sup>4</sup>Wang, C., Wang, H., Li, P., Li, H., Xu, C., Cohen, H., ... & Wu, S. (2020) *The Plant Journal*, 104, 241-251.



SESSION 2  
MICROBIAL GENETICS

## **Evolution and adaptation of *Streptococcus pneumoniae* in the era of expanded conjugate vaccines**

Raquel Sá-Leão

Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA)

Email: [rsaleao@itqb.unl.pt](mailto:rsaleao@itqb.unl.pt)

*Streptococcus pneumoniae* (or pneumococcus) is the main cause of bacterial pneumonia worldwide being associated with significant morbidity and mortality. Pneumococci are often shielded by a polysaccharide capsule which hinders phagocytosis and is considered its main virulence factor. Over 100 capsular types have been described to date and these are the basis of current pneumococcal vaccines. In 2000, the first pneumococcal conjugate vaccine, targeting seven capsular types (7-valent), became available. In 2015, a 13-valent pneumococcal conjugate vaccine was introduced in the Portuguese National Immunization Program. I will discuss the success of these vaccines and how, together with antibiotics, they are shaping the pneumococcal population. I will illustrate how molecular epidemiology and ecology can be used to understand the impact of these interventions and anticipate the effect of future vaccines with expanded valency.



## Unveiling the role of Dps and EndoIII for DNA protection and repair in *Deinococcus radiodurans* upon exposure to genotoxic stress

Guilherme Martins<sup>1</sup>, André A. Gouveia<sup>1</sup>, Sara T. Silva<sup>1</sup>, Filipe Rollo<sup>1</sup>, Ausra Domanska<sup>2</sup>, Sarah Butcher<sup>2</sup>, Elin Moe<sup>1</sup>, Célia V. Romão<sup>1</sup>

<sup>1</sup>ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa. Av. da República, 2780-157 Oeiras, Portugal

<sup>2</sup>Institute of Biotechnology, Helsinki Institute of Life Science, P.O.Box 56 (Viikinkaari 9), FI-00014 University of Helsinki, Finland

Email: [gdc.martins@itqb.unl.pt](mailto:gdc.martins@itqb.unl.pt)

*Deinococcus radiodurans* (*Dr*) is known for showing great resistance to ionizing radiation, desiccation and oxidative stress amongst other extreme conditions<sup>1</sup>. This bacterium possesses different groups of proteins which are involved in the response to several stress agents by either protecting the DNA from suffering damage or repairing efficiently the damaged DNA. In this work we focused on two Dps (DNA binding protein under starved conditions) present in *Dr* (Dps1 and Dps2), which are able to bind and protect DNA against damage<sup>2,3</sup> and on three Endonuclease III proteins (EndoIII-1, EndoIII-2 and EndoIII-3) which are responsible for repairing the damaged DNA through the Base Excision Repair (BER) pathway<sup>4</sup>. To understand the role of these proteins and their possible interplay, we constructed single and double knockout mutants of each of these proteins through the Tripartite Ligation Method, using Overlap PCR, and studied their resistance to stress. The induced stress included exposure to UVC radiation, hydrogen peroxide and methyl viologen, and the stress response was compared with the wild type bacterium. Moreover, the interaction between Dps2 and DNA was studied at molecular and structural levels and we have determined the structure of this complex using Cryo-Electron Microscopy – Single Particle Analysis.

### References

<sup>1</sup>Misra et al (2013) CURRENT SCIENCE, 104, 194-202.

<sup>2</sup>Santos et al (2015) FEBS J, 282, 4307.

<sup>3</sup>Santos et al (2017) JMBiol, 429, 667-687.

<sup>4</sup>Sarre et al (2019) DNA Repair, 78, 45-59.

## Regulatory mechanisms of *yurJ* expression and intra-changeability with MsmX

Inês C. Gonçalves and Isabel Sá-Nogueira

Associate Laboratory i4HB - Institute for Health and Bioeconomy, Applied Molecular Biosciences Unit UCIBIO@REQUIMTE, Laboratory of Microbial Genetics, Department of Life Sciences, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal

Email: [ic.goncalves@fct.unl.pt](mailto:ic.goncalves@fct.unl.pt)

ATP-Binding Cassette (ABC) Transporters are a major group of transporter proteins, present in all domains of life. These transporters rely on ATP hydrolysis for the translocation of molecules across cellular membranes. ABC transporters share the same core structure, which include two Transmembrane Domains (TMDs), embedded in the phospholipidic bilayer and two highly conserved Nucleotide-Binding Domains (NBDs or ATPases), which are responsible for the hydrolysis of ATP and energization of the transporter complex. Our group and others have shown that, unlike what was previously thought, a single ATPase can energize different ABC-type I importers of the Carbohydrate Uptake 1 (CUT1) family. The multitask ATPase MsmX from *Bacillus subtilis* interacts with different ABC sugar importers, however this species contains a second putative ATPase for carbohydrate transport, YurJ. Our group has shown that in its locus YurJ is not able to accumulate and substitute MsmX function, even though its transcript levels are higher than those of *msmX* in the conditions tested. When ectopically expressed, YurJ is able to substitute MsmX in the energization of different carbohydrate importers in an *msmX*-null background. These results prompt us to investigate the hypothesis of an existing genomic context-dependent post transcriptional mechanism is involved in the regulation of *yurJ*. Since *yurJ* is located at the 3'-end of the *frlBONMD* operon and it is co-transcribed with a putative antisense RNA, S1254, we constructed strains with different mutations targeting S1254 and the operon repressor gene *frlR*. These mutations were analysed in both the WT and *msmX*-null genetic background. Our results show that, in the mutant strains, YurJ was able to substitute MsmX in the transport of arabinotriose. Western Blot and RT-qPCR analysis were performed, corroborating the results of growth kinetic parameters. These findings and the implication of YurJ and MsmX intra-exchangeability in *B. subtilis* lifestyle will be discussed.

This work was supported by FCT/MCTES grant nº PTDC/BIA-MIC/30696/2017 to IS-N, grant nº UI/BD/151153/2021 to ICG, grant UIDP/04378/2020 and UIDB/04378/2020 to the Applied Molecular Biosciences Unit – UCIBIO.

## Development of a OMMV-based vector for gene expression in plants

Rafaela Rodrigues Jesus<sup>1</sup>, Patrick Materatski<sup>2</sup>, Maria do Rosário Félix<sup>3</sup>, Maria Doroteia Campos<sup>2</sup>, Mariana Patanita<sup>2</sup>, André Albuquerque<sup>2</sup>, Joana A. Ribeiro<sup>2</sup>, Carla Varanda<sup>2</sup>

<sup>1</sup>Departamento de Química e Bioquímica, Universidade de Évora

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [carlavaranda@uevora.pt](mailto:carlavaranda@uevora.pt)

Viruses are responsible for several important plant diseases; however, they have also been used in biotechnology with different purposes. Many plant viruses have been developed as viral vectors for the production of biopharmaceuticals or for gene expression, with many advantages over the use of transgenic plants. The Alphanecrovirus Olive mild mosaic virus (OMMV) has characteristics that makes it a very promising vector tool. Its small genome makes it easy to manipulate, and it causes only mild systemic symptoms in a wide range of crops. Recently it was developed as a symptomless construct (OMMVp6mutant) which allows its development as vector for a high number of plants.

In this study, a recombinant OMMV was generated by introducing the coding sequence for the GFP (Green fluorescent protein). GFP was placed immediately downstream the OMMVp6mutant coat protein gene. The correct position and orientation of GFP was confirmed through sequencing. The recombinant OMMV was then transcribed, inoculated into *Nicotiana benthamiana* plants and GFP expression levels were observed visually. Quantification was performed through real time PCR. GFP was also introduced into a commercial vector, pk7WG2, using the Gateway technology to compare the efficiency of both systems. The commercial vector carrying GFP was then used to transform *Agrobacterium tumefaciens* C58 and to agroinfiltrate *Nicotiana benthamiana* plants. GFP expression levels were also observed visually and quantified through real time PCR.

Both systems (OMMV and commercial vector) were able to express GFP in plants. Quantification of GFP expression levels in both systems did not reveal any significant differences, showing that OMMV has the ability to work as a viral vector for gene expression in plants.

The wide range of plants OMMV infects and the ease of manipulation places OMMV as a potential viral vector for many applications in biotechnology.

This work was financed through the project ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017, and project ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017, both co-financed by the European Union through the ERDR, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo), ALGARVE 2020 (Regional Operational Program of the Algarve) and through the Foundation for Science and Technology (FCT), in its national component. M.P. was supported by Portuguese National Funds through FCT/MCTES, under the PhD scholarship SFRH/BD/145321/2019, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo. This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.

## Assessment of *Fusarium* spp. and *Magnaportheopsis maydis* in maize in the Iberian Peninsula

Maria Doroteia Campos<sup>1</sup>, Ana Cordeiro<sup>2,3</sup>, Mariana Patanita<sup>1</sup>, Patrick Materatski<sup>1</sup>, Carla M. R. Varanda<sup>1</sup>,  
André Albuquerque<sup>1</sup>, Joana A. Ribeiro<sup>1</sup>, Maria do Rosário Félix<sup>4</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>Escola Superior Agrária de Elvas, Instituto Politécnico de Portalegre, Edifício Quartel do Trem, Avenida 14 de janeiro, nº21  
7350-092 Elvas, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>4</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [mdcc@uevora.pt](mailto:mdcc@uevora.pt)

*Fusarium* spp. and *Magnaportheopsis maydis* are soil-inhabiting fungi that are the causal agents of Fusarium Ear Rot and Late Wilt respectively, two important diseases that can affect maize. Fusarium Ear Rot disease has an increasing relevance due to high production losses, a consequence of the colonization of seeds, roots, and stem by the fungus and can result in mycotoxin-contaminated grains that cause health-threatening issues. There are multiple species of *Fusarium* that affect maize, with the most important being *Fusarium verticillioides*, although climatic conditions and crop management practices influence the occurrence and prevalence of other *Fusarium* species. Late Wilt is characterized by the wilting of maize plants, usually with the onset of symptoms only 70 days after sowing. With the development of the disease, the leaves turn yellow due to lack of water, the stalk becomes hollow and constricted, with dramatic consequences in productivity. Normally, other pathogens are also associated with Late Wilt, namely *F. verticillioides*.

The MED/UÉvora Mycology Laboratory has been recurrently sought after by farmers and seed producers to confirm the presence and to increase the knowledge on *Fusarium* spp. and *M. maydis* in maize field plants, with symptoms of Fusarium Ear Rot and/or Late Wilt. Symptomatic plants from different regions of Portugal and Spain, collected in the years 2019 and 2020, were assessed for *Fusarium* spp. and *M. maydis*. The methodology used, established in the MED/UÉvora Mycology Laboratory was based in real-time PCR (qPCR), using specific TaqMan MGB (Minor Groove Binder) assays. The occurrence of *Fusarium* spp. and *M. maydis* was identified in 41% and 84% respectively of the field samples, and a simultaneous occurrence of both fungi was detected in 36% of the samples. Our study was able to identify both fungi in maize, with diverse origins and with different incidences and a screen of resistant plants.

This work was financed through the project ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017, and project ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017, both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through the FCT in its national component. M.P. was supported by Portuguese National Funds through FCT/MCTES, under the PhD scholarship SFRH/BD/145321/2019, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo. This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.

## Detection and quantification of *Fusarium* spp., the causal agents of tomato root rot and wilt diseases

Carla M. R. Varanda<sup>1</sup>, Mariana Patanita<sup>1</sup>, Joana A. Ribeiro<sup>1</sup>, Maria do Rosário Félix<sup>2</sup>, André Albuquerque<sup>1</sup>, Patrick Materatski<sup>1</sup>, María Doroteia Campos<sup>1</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [mdcc@uevora.pt](mailto:mdcc@uevora.pt)

Tomato, one of the most economically important vegetable crops throughout the world, is affected by a panoply of different pathogens that reduce yield and affect product quality, with the causal agents including fungi, fungus-like organisms, bacteria, viruses and phytoplasmas. Fungal diseases have high impact on tomato production and amongst them we highlight those caused by *Fusarium* spp., as responsible for severe yield losses throughout the world and increasing the carbon footprint caused by the phytosanitary treatments available. Although tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici* is reported as a worldwide destructive disease, other *Fusarium* species have been constantly evolving and have been increasingly associated with many tomato wilt and rot diseases. Given the increasing incidence of *Fusarium* spp. in tomato, the importance of early and accurate detection arises, and highlights the need of precise molecular identification and quantification, offering an additional tool in the screening of resistant plants.

The present study reports the establishment of a methodology for detection and quantification of *Fusarium* spp. genomic DNA (gDNA) in tomato plants, allowing to discriminate *Fusarium* spp. gDNA from other tomato pathogenic fungi. The methodology is based on real-time PCR (qPCR), through the development of a specific TaqMan MGB (Minor Groove Binder) assay, with the selective amplification of the internal transcribed spacer (ITS) region. The work involved the design and application of a specific probe that targets the main pathogenic *Fusarium* spp. (i.e., *F. verticillioides*, *F. oxysporum*, *F. solani*, *F. graminearum*, *F. incarnatum*), that enabled a reliable, sensitive, and reproducible estimation of *Fusarium* spp. accumulation in tomato. The use of qPCR instrumentation combined with the chemistry of TaqMan MGB probes represents the most specific and sensitive detection system when low amounts of target DNA are present, as often occurs in the case of early interactions between plants and fungi.

This work was financed through the project ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017, and project ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017, both co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020, ALGARVE 2020 and through the FCT in its national component. M.P. was supported by Portuguese National Funds through FCT/MCTES, under the PhD scholarship SFRH/BD/145321/2019, co-financed by the European Social Fund through the Regional Operational Program of the Alentejo. J.R. was supported by Portuguese National Funds through Project ALT20-03-0246-FEDER-000056 under scholarship BI\_MESTRE\_Uevora\_CER\_BIOPROTOMATE, co-financed by European Regional Development Fund through Regional Operational Program Alentejo 2020. This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.

## Endophytic microbiomes of susceptible plants to *Xylella fastidiosa* infection

Rita Ramos<sup>1,2</sup>, Alexandra Camelo<sup>2</sup>, Eva Margarida<sup>3,4</sup>, João Trovão<sup>3,4</sup>, Inês Brandão<sup>2,3</sup>, Joana Costa<sup>3,4</sup>,  
Christophe Espírito Santo<sup>2,3</sup>

<sup>1</sup>Universidade da Beira Interior, Faculdade de Ciências, R. Marquês de Ávila e Bolama, 6201-001 Covilhã, Portugal

<sup>2</sup>CATAA – Centro de Apoio Tecnológico Agro-Alimentar, Zona Industrial de Castelo Branco, Rua A, 6000-459 Castelo Branco, Portugal

<sup>3</sup>Universidade de Coimbra, Centre for Functional Ecology, Departamento de Ciências da Vida, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

<sup>4</sup>Laboratório de Fitossanidade, Instituto Pedro Nunes, 3030-199 Coimbra, Portugal

Email: [cespiritosanto@cataa.pt](mailto:cespiritosanto@cataa.pt)

*Xylella fastidiosa* (*Xf*) is considered one of the most dangerous phytopathogenic bacteria in the world. *Xf* can colonize the xylem of numerous agricultural, ornamental, and forestry species and is responsible for a wide range of diseases with enormous economic and environmental impact<sup>1</sup>. The central role of the plant-associated microbiome in maintaining the host's fitness is crucial, however, little is known about the diversity and structure of these communities of endophytic microorganisms<sup>2</sup>. Currently, the search for alternatives for sustainable agriculture is a strategic priority for the control of many plant diseases. Therefore, it is essential to study the diversity of the endophytic microbial community and the interactions between endophytes and plants to understand the biotechnological potential of these microorganisms<sup>3</sup>. In this manner, samples with detected and undetected *Xf* were sequenced for the 16S rRNA gene. The nanopore DNA sequencing library was prepared with the Oxford Nanopore Technologies 16S barcoding kit for DNA sequencing on a MinION Mk1C device. This platform has emerged recently and is, to date, the only portable sequencing platform on the market, which gives it a significant advantage over other third-generation sequencing platforms<sup>4</sup>. In this context, the main objective of this study is to identify and characterize the endophytic microbiome of endemic hosts of *Xf* in Portugal, and to identify possible groups that modulate the infection. Moreover, it is intended to genetically characterize the organism using the MLST approach.

We would like to acknowledge the collaboration of DGAV, ICNF and DRAPN.

### References

- <sup>1</sup>Camino, C., Calderón, R., Parnell, S., Dierkes, H., Chemin, Y., Román-Écija, M., Beck, P. S. A (2021). *Remote Sensing of Environment*, 260, 1-16. 2.
- <sup>2</sup>Morelli, M., García-Madero, J. M., Jos, Á., Saldarelli, P., Dongiovanni, <sup>2</sup>C., Kovacova, M., Saponari, M., Arjona, A. B., Hackl, E., Webb, S., Compant, S. (2021). *Microorganisms*, 9, 1–21.
- <sup>3</sup>Azevedo, J. L., Araújo, W. L., Lacava, P. T. (2016). *Genetics and Molecular Biology*, 39, 476–491.
- <sup>4</sup>Lamb, H. J., Hayes, B. J., Nguyen, L. T., Ross, E. M. (2020). *Genes*, 11, 1–27.

## Evaluation of the effect of tree canopy and dolomitic limestone application on soil microbial activity in the Montado Mediterranean ecosystem

Daniel Bailote<sup>1</sup>, João Serrano<sup>2</sup>, Ana Elisa Rato<sup>3</sup>, Joana A. Ribeiro<sup>1</sup>, Isabel Brito<sup>4</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Biology, School of Science and Technology, University of Évora, Pólo da Mitra, Apartado 94, 7002 – 554 Évora, Portugal

<sup>2</sup>MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Rural Engineering Department, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Plant Science Department, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>4</sup>MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Biology Department, University of Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [m48156@alunos.uevora.pt](mailto:m48156@alunos.uevora.pt)

The *Montado* is an agro-silvo-pastoral ecosystem, explored at several levels. On the tree level it can be made up of oaks like cork oak (*Quercus suber*) and holm oak (*Q. rotundifolia*). The ground cover is occupied by natural biodiverse pastures that are based on poor soils grazed by animals in an extensive regime. One of the major factors affecting the pasture productivity is the soil acidity<sup>1</sup>. The objective of this work is to evaluate the effect of canopy and dolomitic limestone application for soil acidity correction, on soil microbial activity in the *Montado* ecosystem. Soil samples were collected in an experimental field (4ha) located in Mitra Farm in February 2022. The soil samples were subjected to laboratory analysis for the evaluation of different parameters of microbial activity: dehydrogenase,  $\beta$ -glucosidase, phosphatase, arylsulfatase and soil basal respiration. All the measured variables were higher under the tree canopy than outside the tree canopy, and this difference turns out to be smaller in the amended area. These results allow us to perceive the effect of the canopy and soil acidity correction on soil microbial activity in the *Montado* and provide important information regarding the holistic management of this ecosystem. A phylogenetic analysis of the microbiome to test traits driving habitat associations and better understand how microbial communities change over the studied treatments is foreseen.

### References

<sup>1</sup>Serrano J, Shahidian S, Marques da Silva J, Moral F, Carvajal-Ramirez F, Carreira E, Pereira A, Carvalho Md. Evaluation of the Effect of Dolomitic Lime Application on Pastures-Case Study in the Montado Mediterranean Ecosystem. Sustainability. 2020; 12(9):3758. <https://doi.org/10.3390/su12093758>.

## Evolution and diversification of RNAi proteins in Ascomycetes

Pedro Ferreira<sup>1</sup>, Teresa Lino-Neto<sup>1</sup>, Athanasios Dalakouras<sup>1</sup>, Francisca Reis<sup>1</sup>

<sup>1</sup>Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Email: [fpedro17@hotmail.com](mailto:fpedro17@hotmail.com)

RNA interference (RNAi) refers to a eukaryotic mechanism that is responsible for genome protection against mobile genetic elements and defence against virus. Three core components, argonaute (AGO), dicer (DCL) and RNA dependent RNA polymerase (RdRp) are essential for a functional RNAi pathway. In this work, the evolution of argonaute, dicer and RdRp proteins were analysed within 23 Ascomycota species and 5 Basidiomycota species, with special focus on Ascomycota. These three proteins are widely represented in both phyla with a variable number of copies. The present results suggest the occurrence of an ancient duplication of argonaute, dicer and RdRp genes alongside with early diversification of Ascomycota and Basidiomycota. These duplications were followed by additional species-specific diversification. Two main RNAi pathways have been recognized in fungi, Quelling and MSUD. Based on protein architecture and phylogenetic relationship, this work reveals that both pathways are present in the Ascomycota fungi. Furthermore, an apparent evolution between MSUD and the chromatin remodelling pathway present in *Schizosaccharomyces pombe* seems to have occurred. Because all three proteins are present in Ascomycota and their phylogenetic relations are in line with known RNAi pathways, these results suggest a functional RNAi machinery that could be exploited for biotechnological purposes.



## Exploring multi-tissue transcriptomic data for in silico investigation of drugs for the treatment of dengue fever disease

Ana C. Magalhães<sup>1,2,3</sup>, Beatriz Sierra<sup>4</sup>, Daniel Soares<sup>1,2</sup>, Bruno Cavadas<sup>1,2</sup>, Ana B. Perez<sup>4</sup>, Mayling Alvarez<sup>4</sup>, Eglis Aguirre<sup>4</sup>, Claudia Bracho<sup>4</sup>, Maria G. Guzman<sup>4</sup>, Luisa Pereira<sup>1,2</sup>

<sup>1</sup>IS - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

<sup>2</sup>IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal

<sup>3</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

<sup>4</sup>Virology Department, PAHO/WHO Collaborating Center for the Study of Dengue and Its Vector, Pedro Kourí Institute of Tropical Medicine (IPK), Havana 11400, Cuba

Email: [acmagalhaes@ipatimup.pt](mailto:acmagalhaes@ipatimup.pt)

Multi-omics data such as transcriptomics, proteomics and pathogen-host interactomics, are being explored for the in silico-informed selection of drugs, prior to the complex and expensive functional evaluation of drugs. The effectiveness of this kind of strategy has been put to the test in the current COVID-19 pandemic, leading to a few drugs being rapidly repurposed as treatment against SARS-CoV-2 infection. Several neglected tropical diseases, for which treatment remains unavailable, as Dengue fever disease, would also benefit from the informed in silico investigations of drugs.

In this work, we aimed to identify drugs that could be effective in the treatment of Dengue disease, by applying a multi-tissue transcriptomic-informed in silico approach. First, we analyzed the transcriptome from liver (published data), spleen (our own data) and blood (published), and checked for molecular pathways significantly changed. Second, we evaluated through the CMap tool which drugs could interfere with the expression profiles from each of the tissues in the Dengue context.

We identified some heterogeneity in the molecular pathways between tissues, reflecting tissue specialization. This dissimilarity could initially imply that a cocktail-based treatment would be more suitable, however, given the high connectivity between molecular networks and the multi-effects of drugs, we identified candidate drugs that can be effective across the various tissues for the treatment of Dengue disease. In fact, the common mechanisms of action “Adrenergic receptor antagonist”, “ATPase inhibitor”, “NF-kB pathway inhibitor” and “Serotonin receptor antagonist”, were identified as druggable (e.g., with oxprenolol, digoxin, auranofin and palonosetron, respectively) to oppose the effects of severe Dengue infection in all the analysed tissues. These are good candidates for future functional evaluation and clinical trials.

## Functional characterization of parasitism-related genes from *Bursaphelenchus xylophilus* may suggest involvement on the nematode oxidative stress response

Marina Curto<sup>1</sup>, Cláudia Vicente<sup>2</sup>, Margarida Espada<sup>2</sup>

<sup>1</sup>Universidade de Évora, Évora, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies, and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [marina\\_curt@hotmail.com](mailto:marina_curt@hotmail.com)

The pinewood nematode *Bursaphelenchus xylophilus* is a migratory plant-parasitic nematode that infects conifer pine trees (mainly *Pinus* species) causing the pine wilt disease. In Portugal, the maritime pine, *Pinus pinaster*, is the most susceptible conifer species, and its decline has ecological and economic impact on forest ecosystems and wood industry<sup>1</sup>. Functional studies suggest that effectors are one key part of a multi-layered detoxification strategy deployed by *B. xylophilus* in order to protect itself from host defense responses. *B. xylophilus* secretes detoxification enzymes into the host, while simultaneously upregulating other detoxification enzymes within its digestive system<sup>2</sup>.

Previous transcriptomic data from pre-parasitic and parasitic stages revealed differentially expressed genes during plant infection<sup>2</sup>. In the present study, a list of candidate genes previously identified in the transcripts of a specialized tissue (pharyngeal gland cells) involved in the nematode parasitism were selected for functional characterization under ROS conditions. *In silico* analysis of all candidate genes showed that these genes putatively encode for proteins with signal peptide and no transmembrane domain, suggesting secretion during the infection. In the oxidative stress assays<sup>4</sup>, some of these candidate genes were upregulated after 24h exposure to a natural oxidative agent (hydrogen peroxide) stimulus, in comparison with the expression of the antioxidant enzyme catalase, which directly degrades hydrogen peroxide to water and oxygen. Overall, our results suggest that some of these genes might have a potential role in ROS scavenging activity during plant parasitism of *B. xylophilus*.

### References

<sup>1</sup>Vicente CSL, Espada M, Vieira P, Mota M (2012) *Eur J Plant Pathol* 133:89-99.

<sup>2</sup>Espada M, Silva, A.C., Eves-van den Akker S, Cock PJA., Mota M, Jones JT (2016) *Mol Plant Pathol* 17: 286-295.

<sup>3</sup>Espada M, Eves-van den Akker S, Maier T, Paramasivan V, Baum T, Mota M, Jones JT (2018) *BMC Genomics* 19:553.

<sup>4</sup>Vicente CSL, Ikuyo Y, Shinya R, Mota M, Hasegawa K (2015) *PLoS ONE* 10: e0123839.

## Genomic analysis of *Sinorhizobium meliloti* IRAMC:0087 an halotolerant rhizobium isolated from the Tunisian desert

Roukaya Ben Gaied<sup>1</sup>, Imed Sbissi<sup>1</sup>, Clarisse Brígido<sup>2</sup>, Mohamed Tarhouni<sup>1</sup>

<sup>1</sup>Arid Lands Institute of Médenine, Pastoral Ecology Laboratory University of Gabés- Tunisia

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [roukaya.bengaied@gmail.com](mailto:roukaya.bengaied@gmail.com)

Arid and Saharan regions of Tunisia have great interest due to their endemic spontaneous legume's diversity. The strain IRAMC:0087 was isolated from root nodules of a Saharan shrub *Genista saharae* growing in Southern Tunisia. Phenotypic characterization of this strain has revealed tolerance to high salinity levels, drought and high temperatures. To investigate the basis of this, we sequenced its complete genome and compared it to the genome of the closely related strain *Sinorhizobium meliloti* NBRC 14782<sup>T</sup>, an alfalfa (*Medicago sativa*) microsymbiont. The genome comprises 7,265,739 bp, which is comparable in size to other *S. meliloti* strains and contains a GC content of 61.94%. In total, 7536 protein-encoding sequences, 51 tRNAs and 5 rRNAs were identified. The genome encodes gene clusters supporting rhizosphere processes, secondary bioactive metabolites, plant growth-promoting activities and symbiosis. Gene distribution into COG functional categories revealed that the percentage of genes was assigned to amino acid transport and metabolism (10.92%), general function prediction (10.5%), carbohydrate transport and metabolism (9.52%), and transcription (9.21%). Despite the difference in size, IRAMC:0087 and NBRC 14782<sup>T</sup> genomes present a similar relative occurrence of functional protein encoding genes and do not show any gross genomic alterations. Interestingly, although IRAMC:0087 contains more protein encoding genes than NBRC 14782<sup>T</sup> (7536 vs 6696 genes), the major difference lies in the number of not functionally classifiable genes. Nevertheless, the number of genes for transposable elements (9 vs 2), for managing membrane transport (205 vs 147 genes) and for conducting iron acquisition and metabolism (41 vs 31 genes) are higher in IRAMC:0087 than NBRC 14782<sup>T</sup>. IRAMC:0087 is an interesting strain as it exhibits an endophytic and symbiotic behavior with hosts adapted to extreme climatic conditions. Comparative genomic analyses with other rhizobial strains have the potential to reveal novel factors mediating symbiosis under those conditions.

## Impact of the stringent stress response on the expression of oxacillin resistance in staphylococcal strains carrying *mecA*, *mecA1* or *mecC* determinants

Catarina Milheiriço<sup>1</sup>, Alexander Tomasz<sup>2</sup> and Hermínia de Lencastre<sup>1,2</sup>

<sup>1</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Oeiras, Portugal

<sup>2</sup>Laboratory of Microbiology & Infectious Diseases, The Rockefeller University, New York, NY, USA

Email: [cicm@itqb.unl.pt](mailto:cicm@itqb.unl.pt)

Acquisition of *mecA* gene by *Staphylococcus aureus* is of major clinical importance since it confers a resistant phenotype to virtually the entire large family of structurally diverse  $\beta$ -lactam antibiotics. While the resistance determinant *mecA* is essential, optimal expression of the resistance phenotype requires additional factors as well. Previous studies showed that the great majority of clinical isolates of methicillin-resistant *S. aureus* (MRSA) have a heterogeneous resistant phenotype and we observed that strains carrying methicillin genetic determinants other than *mecA* also produce similar heterogeneous phenotypes. All these strains were able to express high and homogeneous levels of oxacillin resistance when sub-inhibitory concentrations of mupirocin, an effector of the stringent stress response, was added to the growth media. These observations led us to propose that a common molecular mechanism for the establishment of high and homogeneous oxacillin resistance must be present among isolates carrying different methicillin resistance determinants.

In this work, we tested this hypothesis by using whole-genome sequencing (WGS) to compare isogenic populations differing only in their degrees of oxacillin resistance and carrying various methicillin genetic determinants – *mecA*, *mecC* or *mecA1*. Although no common mutated genes were found in the 15 oxacillin H<sup>\*</sup>R derivatives (representing the three genetic backgrounds in study), in all of the isolates we were able to detect the presence of mutated genes related to guanine metabolism and consequently to stringent stress response. These genetic determinants – *hpt*, *relA2* and *guaB* – were previously reported as implicated in H<sup>\*</sup>R phenotypes in MRSA subpopulations belonging to different genetic backgrounds, strengthening the evidence for the involvement of the stringent stress response in defining the antibiotic resistance level, regardless of clonal type or the methicillin genetic determinant the strains carried. Additional mutations that may impair protein biosynthesis have also been identified, a phenomenon also known to occur when the stringent stress response is activated.

## Lanthipeptides and haloazolisins: competition peptides from haloarchaea?

Thales Viana<sup>1</sup>, Elena Cassin<sup>1</sup>, Ana Catarina Moreirinha<sup>2</sup>, Sónia Mendo<sup>1</sup>, Tânia Caetano<sup>1</sup>

<sup>1</sup>CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal

<sup>2</sup>CESAM and Department of Chemistry, University of Aveiro, Aveiro, Portugal

Email: [thalesviana@ua.pt](mailto:thalesviana@ua.pt)

Ribossomally synthesized and post-translationally modified peptides (RiPPs) are secondary metabolites (SMs) which often display antimicrobial activity and are encoded by biosynthetic gene clusters found in the genome of many prokaryotes. Such peptides have been characterized mainly from bacterial producers. RiPPs biosynthesized by organisms of the Archaea domain are still poorly investigated despite the discovery of several RiPPs clusters in their genomes, especially in haloarchaea<sup>1</sup>. Our model organism, *Haloferax mediterranei* 33500, possesses two of these clusters that putatively encode the production of lanthipeptides and haloazolisins (a type of TOMM peptide)<sup>2</sup>. The first is a well characterized group that contains lanthionines and methylanthionines amino acids<sup>3</sup>, while the second group is still poorly studied. Additionally, *H. mediterranei* 33500 strain exhibits antimicrobial activity, although its molecular nature was not elucidated so far. Our objective was to understand if in *H. mediterranei* this activity is encoded by the two RiPPs clusters, which may represent an ecological advantage for haloarchaea. To do so, first we analysed the transcription level of selected genes (encoding the core biosynthetic enzymes [CBE]) by qPCR along 4 days. Results suggest these are not cryptic, as observed for many bacterial SMs clusters. Then, the pop-in/pop-out strategy was applied to generate knockout mutants for the CBE genes (*lanM1*, *lanM2* and *lanM3* for lanthipeptides and *ycaO* for haloazolisins). The bioactivity of the mutants was tested and found to be unaffected, suggesting that the bioactivity detected in *H. mediterranei* is not associated with these RiPPs. To further understand if these proteins are related with other cellular processes, the mutants will be further investigated.

### References

- <sup>1</sup>Malit, J., Wu, C., Liu, L. L., & Qian, P. Y. (2021). Global Genome Mining Reveals the Distribution of Diverse Thioamidated RiPP Biosynthesis Gene Clusters. *Frontiers in microbiology*, 12, 635389. <https://doi.org/10.3389/fmicb.2021.635389>.
- <sup>2</sup>Castro, I., Costa, H., Turgeman-Grott, I., Allers, T., Mendo, S., & Caetano, T. (2021). The lanthipeptide biosynthetic clusters of the domain Archaea. *Microbiological research*, 253, 126884. <https://doi.org/10.1016/j.micres.2021.126884>.
- <sup>3</sup>Lagedroste, M., Reiners, J., Knospe, C. V., Smits, S., & Schmitt, L. (2020). A Structural View on the Maturation of Lanthipeptides. *Frontiers in microbiology*, 11, 1183. <https://doi.org/10.3389/fmicb.2020.01183>.

## Metabarcoding of the fungal and bacterial communities present in a compost of olive pruning residues

André Albuquerque<sup>1</sup>, Mariana Patanita<sup>1</sup>, Carla M. R. Varanda<sup>1</sup>, Maria Doroteia Campos<sup>1</sup>, Patrick Materatski<sup>1</sup>, Joana A. Ribeiro<sup>1</sup>, António Bento Dias<sup>2</sup>, Maria do Rosário Félix<sup>3</sup>

<sup>1</sup>MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, 7006-554 Évora, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Engenharia Rural, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, 7006-554 Évora, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, 7006-554 Évora, Portugal

Email: [andrealb@uevora.pt](mailto:andrealb@uevora.pt)

The increasing area of olive orchards in Alentejo create a management concern regarding the amount of agrowastes generated. Composting is a process of recycling decomposing organic matter by turning animal and plant wastes into soil fertilizers, reducing the need to use commercial ones. Nevertheless, it is still not clear how the microbiological populations change, particularly the phytopathogens. The tested compost in our study was a mixture of sheep manure together with dead leaves and small branches from olive pruning. The composting method used was the aerated windrow in order to maintain humidity levels between 40 to 60%. Maturation of the compost was considered after four months. Samples were collected from: the initial vegetal component obtained at 30 and 50 cm of depth (AV30/AV50); the sheep manure; the compost at two months and at the final composting time. Genomic DNA was extracted and sent for next-generation sequencing using the 16S and ITS regions to respectively identify the bacterial and fungal communities present. The results obtained showed a shift in the microbial composition of the final compost when comparing to the individual composting components and intermediate composting stage. Overall, the final composting stage presented the lowest abundance of bacteria but attained the highest bacterial and fungal diversities (425 and 235 genera/species, respectively), suggesting the introduction of beneficial microbe communities that assist pathogen suppression in the soil and promote plant growth. Several thermophilic organisms were among the most abundant taxa found in the final compost, including *Thermomyces lanuginosus* and *Mycothermus thermophilus* (top 1%). The applied composting strategy drastically decreased the abundance of known fungi with phytopathogenic activity such as *Penicillium* spp. and *Pyrenochaeta* spp. which were the most abundant in AV30 and AV50, respectively. Furthermore, the presence of *Acidovorax* spp., *Williamsia* spp., *Xanthomonas* spp. and *Chryseobacterium* spp. was eliminated from the composting samples.

This work was supported by the project PDR2020-101-031764.

## Metagenomic data from symptomatic grapevines affected by Grapevine Trunk Diseases shows a variable fungal fraction between cultivars in Douro Region

Filipe Azevedo-Nogueira<sup>1,2</sup>, Ana Gaspar<sup>3</sup>, Cecília Rego<sup>3</sup>, Sara Barrias<sup>1,2</sup>, Juliana Lopes<sup>1,2</sup>, Helena Gonçalves<sup>4</sup>, Ana Margarida Fortes<sup>2</sup>, Aleš Eichmeier<sup>5</sup>, Kateřina Štůsková<sup>5</sup>, David Gramaje<sup>6</sup>, Paula Martins-Lopes<sup>1,2</sup>

<sup>1</sup>University of Trás-os-Montes and Alto Douro, School of Life Science and Environment, DNA &RNA Sensing Lab, Vila Real, Portugal

<sup>2</sup>BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

<sup>3</sup>LEAF - Linking Landscape, Environment, Agriculture and Food-Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal

<sup>4</sup>REQUIMTE, Instituto Superior de Engenharia do Porto, Porto, Portugal

<sup>5</sup>Mendeleum – Institute of Genetics, Mendel University in Brno, Lednice, Czech Republic

<sup>6</sup>Institute of Grapevine and Wine Sciences (ICVV), Spanish National Research Council (CSIC), Logroño, Spain

Email: [plopes@utad.pt](mailto:plopes@utad.pt) ; [filipem.a.nogueira@gmail.com](mailto:filipem.a.nogueira@gmail.com)

Wine production is deeply entangled in Portugal's economy and culture, which is one of the most relevant wine-producing countries<sup>1</sup>. Higher grape yields and quality are achievable when the vineyards are submitted to good phytosanitary practices. Therefore, the control of biotic stresses is a major concern in vineyard management. Grapevine Trunk Diseases (GTDs) are fungal infections that affect mostly vine woody tissues, and lead to a reduced vine vigour, production, longevity and, ultimately, death, representing a major viticulture constraint<sup>2</sup>. The aim of this work was to understand the fungal diversity associated with two cultivars of grapevine exhibiting symptoms of GTDs in the Douro region. A total of 15 wood samples belonging to Aragonez cultivar (n=7) and Touriga Nacional cultivar (n=8) were collected in different vineyards of the Douro region. DNA samples were extracted using FastDNA® Spin Kit (MP Biomedicals, USA) and were sequenced in a NovaSeq PE250 flow cell (Illumina, San Diego, USA), targeting the ITS region<sup>3</sup>. Our results indicate a possible host-pathogen correlation between some fungal taxa and grapevine cultivars with *Phaeoacremonium* and *Phaeoconiella* genera being more represented in Aragonez samples, whereas Touriga Nacional samples show higher prevalence of *Neofusicoccum* and *Diaporthe* genera. Overall, these results allow us to better understand the lifestyle of GTDs pathogens in the Douro region and how this may vary within genotypes, which may assist, in the future, grape producers in the implementation of more efficient vineyard protection measures.

This work was financed through the FCT Project TrunkBioCode ref. PTDC/BAA-DIG/1079/2020 and PhD fellow to F.A.-N. Ref. 2020.04459.BD

### References

- <sup>1</sup>Pereira et al., (2018). *Beverages*, 4, 71.  
<sup>2</sup>Gramaje et al., (2018). *Plant Disease*, 102(1), 12-39.  
<sup>3</sup>Martínez-Diz et al., (2020). *Fungal Ecology*, 48, 100994.

## Niche-specific genes of vaginal *Candida albicans* isolates: an in-silico analysis

Mariana Fernandes<sup>1,2</sup>, Carlos Gaspar<sup>1,2,3</sup>, Michèle Claire Breton<sup>1</sup>, José Martinez-de-Oliveira<sup>1</sup>, Ana Palmeira-de-Oliveira<sup>1,2,3</sup>, Joana Rolo<sup>1</sup>

<sup>1</sup>CICS-UBI: Health Sciences Research Center, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal

<sup>2</sup>Faculdade de Ciências da Saúde, University of Beira Interior, Covilhã, Portugal

<sup>3</sup>Labfit-HPRD:Health Products Research and Development Lda, Covilhã, Portugal

Email: [joanarolo@fcsaude.ubi.pt](mailto:joanarolo@fcsaude.ubi.pt)

Niche-specific genes can be found in the accessory genome of many pathogens. By performing a comparative genomic analysis between isolates collected from different host sites, it could be possible to identify genes implicated in the colonization and infection of those niches<sup>1</sup>. In this study, this analysis was applied to the fungus *Candida albicans*, a colonizer and pathogen of several human tissues. The genomes of 23 *Candida albicans* clinical isolates were obtained from the NCBI Datasets, 6 from the oral cavity, 2 from the vagina, and 15 from blood. The genes were predicted for all isolates, and a pangenome was constructed, characterizing the accessory and core genome. A maximum-likelihood phylogenetic tree was also constructed, and the MLST profiles were characterized. From the accessory genome, the presence/absence of protein-coding genes between the isolates was investigated, focusing on the genes more prevalent in the vaginal isolates. Lastly, a genome-scale protein function classification was applied.

Of the 5810 genes encoding protein families detected, 83.7% were present in all isolates. In the obtained phylogenetic tree, most clusters had isolates with matches in their MLST profiles, with one or several alleles in common. Furthermore, a possible association between the collection site and the genomic relatedness of the isolates was found, with isolates from specific niches notably segregating together, namely the vaginal and oral isolates contrasting with the blood isolates. Of the 10 comparably more prevalent protein-coding genes in the vaginal isolates, 9 had no previous functional annotation. An additional analysis focusing on the predicted protein domains allowed the annotation of three genes: a 3'(2'),5'-bisphosphate nucleotidase, a Pumilio-family RNA binding repeat, and an isocitrate/isopropylmalate dehydrogenase. Our analysis identified vaginal-specific genes, some possibly associated with metabolism, but further studies are needed regarding the functional annotation of differently distributed genes.

### References

<sup>1</sup>Guimarães, L. C., Florczak-Wyspianska, J., de Jesus, L. B., Viana, M. V., Silva, A., Ramos, R. T., Soares, S., & Soares, S. (2015). Inside the Pan-genome - Methods and Software Overview. *Current genomics*, 16(4), 245–252. <https://doi.org/10.2174/1389202916666150423002311>.



## Occurrence of diploid nuclei throughout the life cycles of Pucciniales fungi disclosed by cytogenomic approaches

Rita Carvalho<sup>1</sup>, Helena G. Azinheira<sup>1,2</sup>, Teresa Ribeiro<sup>1</sup>, Sílvia Tavares<sup>2,3</sup>, Marta Monteiro<sup>4</sup>, Maria do Céu Silva<sup>1,2</sup>, Leonor Morais-Cecílio<sup>1</sup>, Pedro Talhinhos<sup>1</sup>

<sup>1</sup>Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal

<sup>2</sup>Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

<sup>3</sup> Department of Plant and Environmental Sciences, Section for Plant and Soil Science, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

<sup>4</sup> IGC, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Email: [ritamdcarvalho@isa.ulisboa.pt](mailto:ritamdcarvalho@isa.ulisboa.pt)

Pucciniales (Basidiomycota) is the most speciose order of phytopathogens. Rust fungi are biotrophs and affect a phylogenetic distinct range of hosts. Pucciniales fungi establish highly specific and complex interactions with their hosts.

Flow Cytometry (FCM) had revealed the genome size of Pucciniales fungi (353 Mbp, in average) to be approximately six times larger than the average fungal genome size (61.6 Mbp). In this work, we present another unexpected discovery revealed by FCM. Diploid nuclei were observed in diverse life stages of rust fungi described as being only haploid. Rust fungi from 37 species were analysed by FCM, in diverse life stages, and it was found, in addition to the 1C peak, a large 2C peak. In most cases, a 4C peak is also noticeable, indicating that diploid nuclei are actively replicating. Nuclei from ten rust fungi were also analysed by FISH using rDNA probes. FISH evaluation allowed to determine the ploidy of each nucleus under analysis and confirmed the FCM results.

These findings challenge all mycology literature that allege these fungi are haploid during most of their life cycles. The causes underlying this phenomenon are not clear. Thus, future studies are required to clarify the nature of this haploid/diploid phenomenon that will lead to a better understand of Pucciniales fungi.

## Olive fruit fly symbionts persisting the impact of metamorphosis: their role is yet to be discovered

Catarina Campos<sup>1</sup>, Luís Gomes<sup>2</sup>, Fernando Rei<sup>3</sup>, Tânia Nobre<sup>3</sup>

<sup>1</sup>Laboratory of Molecular Biology, MED - Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup> MED - Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup> Laboratory of Entomology, MED - Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Email: [tnobre@uevora.pt](mailto:tnobre@uevora.pt)

The current symbiotic view of the organisms also calls for new approaches in the way we perceive and manage our pest species. The olive fruit fly, the most important olive tree pest, is dependent on an obligate bacterial symbiont for its larvae development in the immature fruit. This symbiont, *Ca. Erwinia dacicola*, is prevalent through olive fruit fly life stages and promotes the detoxification of oleuropein, the main phenolic compound on the immature olive fruit. In this work, we have examined the *Ca. Erwinia dacicola* numbers in larvae, pupae, and adults retrieved from two olive tree cultivars ('Galega' and 'Redondil'), via a targeted real-time PCR (RT-qPCR) approach on the 16S sequence. Furthermore, we analysed the complete bacterial microbiota present in the olive fruit fly at these three development stages *via* 16S metabarcoding. RT-qPCR results showed that the numbers of *Ca. E. dacicola* varied amongst developmental stages, with highest values at the larval stage, followed by a drastic drop at the pupae, and then a slight recovery at the adult stage. Results from metabarcoding revealed that besides the large presence of *Ca. E. dacicola*, the olive fruit fly harbors a diverse bacterial flora of which 13 OTUs were determined to persist even throughout metamorphosis (*Corynebacterium* sp., *Delftia* sp., *Enhydrobacter* sp., *Kocuria* sp., *Micrococcus* sp., *Propionibacterium* sp., *Pseudomonas* sp., *Raoultella* sp. and *Staphylococcus* sp.). Our results show that the microbial community associated to *olive fruit fly* is subjected to substantial changes across developmental stages, which suggests a role in fulfilling specific needs during insects' development. A clear understanding of the mechanisms underlining changes in microbiota composition in the olive fruit fly is important to understand the biology of insect pests. These findings open a new window of opportunities in symbiosis-based pest management.

This work is supported by National Funds through FCT - Foundation for Science and Technology under the research project PTDC/ASP-PLA/30650/2017.

## RNase R, a key factor for *Streptococcus pneumoniae* biofilm formation

André Alípio Santos<sup>1</sup>, Cátia Bárria<sup>1</sup>, Cecília Arraiano<sup>1</sup>; Susana Domingues<sup>1</sup>

<sup>1</sup>Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Lisboa, Portugal

Email: [andre.f.a.santos@itqb.unl.pt](mailto:andre.f.a.santos@itqb.unl.pt)

*Streptococcus pneumoniae* is the leading cause of pneumonia and meningitis. The prevalence of strains with serotypes not included in the existing vaccines is a problem. Furthermore, the increasing antibiotic resistance led the World Health Organization to include pneumococcus in the list of the twelve priority pathogens which urgently require new antimicrobials.

*S. pneumoniae* is an opportunistic pathogen, often colonizing the human upper respiratory tract. Both asymptomatic colonization of the nasopharynx and infection of deeper tissues require biofilm production, a crucial step for infections by *S. pneumoniae*. During infection, adaptation to stress conditions inside the host requires the modulation of the levels of several bacterial transcripts. Ribonucleases play a crucial role in the control of gene expression and they have been implicated in stress adaptation and virulence.

RNase R is the only 3′–5′exoribonuclease able to degrade highly structured RNAs. We have shown that the absence of this important RNase in *S. pneumoniae* strongly affects the ability of this bacterium to cause infection<sup>1</sup>. Our results indicate a role for RNase R in biofilm formation and the absence of this ribonuclease biofilm production is strongly reduced. Since adhesion is the first step in biofilm formation, we have studied the influence of RNase R in adhesion factors, namely the mRNAs of the major pneumococcal autolysin *lytA* were affected. Fatty Acid membrane composition is also important for biofilm formation and RNase R seems to also control the expression of some transcripts from the fatty acid biosynthesis pathway.

Our results show that *S. pneumoniae* RNase R is important for biofilm production, and it can be used to design new therapies for intervention.

This work was financed through the FCT Project (EXPL/BIA-MOL/1244/2021)

### References

<sup>1</sup>Bárria, C.; Mil-Homens, D.; Pinto, S.N.; Fialho, A.M.; Arraiano, C.M.; Domingues, S. *RNase R, a New Virulence Determinant of Streptococcus pneumoniae*. *Microorganisms* 2022, 10, 317. <https://doi.org/10.3390/microorganisms10020317>.

## Size doesn't matter: a new signature for the Carbohydrate Uptake 1 (CUT1) family?

Lia M. Godinho, Catarina Alves, Joana Almeida, Isabel de Sá-Nogueira

Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal

Applied Molecular Biosciences Unit UCIBIO@REQUIMTE, Laboratory of Microbial Genetics, Department of Life Sciences, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

Email: [isn@fct.unl.pt](mailto:isn@fct.unl.pt)

Type I ABC transporters, present in prokaryotic and plant cells, share a modular architecture: a substrate binding domain (SBP), two hydrophobic transmembrane domains (TMDs) and two cytoplasmic nucleotide binding domains (NBD) with highly conserved sequence motifs, that bind and couple ATP hydrolysis to the transport process<sup>1</sup>. The hydrophobic transmembrane domains of ABC importers are further categorized into two subfamilies, carbohydrate uptake transporter family 1 (CUT-1), importing oligosaccharides, and carbohydrate uptake transporter family 2 (CUT-2), translocating monosaccharides<sup>2</sup>. In our lab, while studying a group of distinct ABC-type I importers of the CUT1 family from *Bacillus subtilis*, which are energized by a single multitask ATPase MsmX, it was evident the presence of a highly conserved C-terminal sequence at the end of the second (shorter) TMD<sup>3</sup>.

In this work, the AraNPQ-MsmX importer system from *B. subtilis*, was subjected to mutagenic analysis and characterization of protein-protein interactions. The combined data, obtained in *E. coli* using a bacterial two-hybrid system and co-immunoprecipitation assays in *B. subtilis*, revealed that although not crucial for contacts between TMD and NBD the small C-terminal tail (GVKKG) is necessary for proper complex assembly and subsequent substrate transport.

This work was supported by FCT/MCTES grant nº PTDC/BIA-MIC/30696/2017 to IS-N, grant nº UI/BD/151153/2021 to ICG, grant UIDP/04378/2020 and UIDB/04378/2020 to the Applied Molecular Biosciences Unit – UCIBIO.

### References

- <sup>1</sup>Locher, K. P. (2016). Mechanistic diversity in ATP-binding cassette (ABC) transporters. *Nature structural & molecular biology*, 23(6), 487-493.
- <sup>2</sup>Saier, M. H. (2000). A functional-phylogenetic classification system for transmembrane solute transporters. *Microbiol. Mol. Biol. Rev.* 64, 354–411.
- <sup>3</sup>Ferreira, M. J., Mendes, A. L., & de Sá-Nogueira, I. (2017). The MsmX ATPase plays a crucial role in pectin mobilization by *Bacillus subtilis*. *PLoS one*, 12(12), e0189483.

## Spatial characterization of soil microbial activity in a vineyard

Inês Vieira<sup>1</sup>, Filipa Tereso<sup>2</sup>, Pedro Tereso<sup>2</sup>, Ana Alexandre<sup>3</sup>, Isabel Brito<sup>3</sup>

<sup>1</sup>MED - Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Pólo da Mitra, Apartado 94, 7002 – 554 Évora, Portugal

<sup>2</sup>Agrosustentável, Centro de Incubação ANJE, Rua Fernanda Seno n.6, 7005 – 485 Évora, Portugal

<sup>3</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Biology, School of Science and Technology, University of Évora, Pólo da Mitra, Apartado 94, 7002 – 554 Évora, Portugal

Email: [m44705@alunos.uevora.pt](mailto:m44705@alunos.uevora.pt)

At the beginning of the 20<sup>th</sup> century, agricultural production increased with a high cost to the environment due to the replacement of diversified and balanced ecosystems by highly simplified ones, and to the use of agronomic practices that cause soil erosion, contamination of reservoirs and water lines, emission of greenhouse gases, the spread of new pathogens, among others<sup>1</sup>. Because soil is a natural resource where numerous biological processes that are fundamental for the ecological balance take place, its preservation and proper management is extremely important<sup>2</sup>. Soil quality and plants comfort are closely related to the soil microbiome and its activity. Monitoring the spatial variation of microbial activity, together with other variables at the level of the vine, allow us to assess possible cause-effect relationships that will contribute to improve the management of the vine agroecosystem. In order to obtain relevant information that contributes to a better knowledge of the vineyard soil microbiome activity, the microbial biomass and microbial respiration were quantified. The activity of important enzymes, such as dehydrogenase, arylsulfatase,  $\beta$ -glucosidase and phosphatase was also assessed. The analysis of results using QGIS (a free and open source geographic information system) allowed the identification of areas with similar patterns regarding enzymatic activity and ultimately spatially relate different parameters. This information can support differential agronomic management in the vineyard to prevail over specific problems and grant more sustainable approaches.

### References

<sup>1</sup>Rosset, J. S., Coelho, G. F., Greco, M., Strey, L., & Gonçalves Junior, A. C. (2014). Agricultura Convencional versus Sistemas Agroecológicos: Modelos, Impactos, Avaliação da Qualidade e Perspectivas. *Scientia Agraria Paranaensis*, 13(2), 80–94. <https://doi.org/10.18188/1983-1471/sap.v13n2p80-94>.

<sup>2</sup>Komatsuzaki, M., & Ohta, H. (2007). Soil management practices for sustainable agro-ecosystems. *Sustainability Science*, 2(1), 103–120. <https://doi.org/10.1007/s11625-006-0014-5>.

## Study of an NDM-producing *Klebsiella pneumoniae* outbreak in a Portuguese hospital approached from a genetic perspective

Sara Silva<sup>1,2,3</sup>, Adriana Ribeiro<sup>3</sup>, Vanessa Oliveira<sup>2</sup>, Elmano Ramalheira<sup>3</sup>, Sónia Mendo<sup>2</sup>, Sónia Ferreira<sup>3,4</sup>,  
Tânia Caetano<sup>2</sup>

<sup>1</sup>Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

<sup>2</sup>CESAM & Department of Biology, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

<sup>3</sup>Centro Hospitalar do Baixo-Vouga, EPE, Aveiro, Portugal

<sup>4</sup>Department of Medical Sciences, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

Email: [saramssilva@ua.pt](mailto:saramssilva@ua.pt)

The emergence and spread of antibiotic-resistant bacteria is a global public health problem with serious clinical and economic implications<sup>1</sup>. *Klebsiella pneumoniae* (ESKAPE microorganism)<sup>2</sup> is an example of multidrug-resistant bacteria responsible for nosocomial bacterial infections associated with high mortality and morbidity<sup>3</sup>, also showing paradigms of pathogenesis, transmission, and resistance<sup>4</sup>, with the ability to escape the antibiotics action<sup>2</sup>. *K. pneumoniae* is the main host of the *bla<sub>NDM</sub>* gene<sup>5</sup>, which encodes the New Delhi metallo-beta-lactamase (NDM), a beta-lactamase (BL) able to hydrolyse most beta-lactam antibiotics, mainly carbapenems<sup>6</sup>, which has no effective inhibitors<sup>5</sup>. The *bla<sub>NDM</sub>* gene is frequently associated with mobile genetic elements<sup>6</sup> and other antibiotic resistance genes, which increases its pathogenicity<sup>5</sup>. Therefore, the presence of NDM-producing *K. pneumoniae* (NDM-p-KP) constitutes a serious health problem, especially when they are found in hospitals and healthcare units, where a considerable proportion of immunocompromised individuals and/or with associated diseases are found.

The aim of this work was to monitor the sudden increase of NDM-p-KP strains, from August to September of 2021, at Centro Hospitalar do Baixo-Vouga and to study, at the genetic level, the eight NDM-p-KP carbapenem strains, collected from urine samples of infected patients. For this, we determined their epidemiological relationship and typing group, the NDM variant and identified the *bla<sub>NDM</sub>* genetic environment and possible virulence factors. The different methodologies used included PCR, PFGE and genome sequencing. Results demonstrate that the *bla<sub>NDM</sub>* gene is probably located on the chromosome, rather than in plasmids. PCR and PFGE results show a high-level of similarity between strains (> 90%), which will be confirmed by genome sequencing. The last will also allow to identify virulence factors that might promote the pathogenicity of NDM-p-KP strains.

### References

- <sup>1</sup>Loureiro, R. J., Roque, F., Teixeira Rodrigues, A., Herdeiro, M. T., Ramalheira, E. (2016). *Rev. Port. Saúde Pública*, 34, 77–84.
- <sup>2</sup>Wyres, K. L. & Holt, K. E. (2018). *Curr. Opin. Microbiol.*, 45, 131–139.
- <sup>3</sup>Riquelme, S. A., Ahn, D. & Prince, A. (2018). *J. Innate Immun.*, 10, 442–454.
- <sup>4</sup>Rice, L. B. (2008). *J. Infect. Dis.*, 197, 1079–1081.
- <sup>5</sup>Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., Zong, Z. (2019). *Clin. Microbiol. Rev.* 32, 1-45.
- <sup>6</sup>Moellering, R. C. (2010). *N. Engl. J. Med.* 363, 2377–2379.

## The genetics of OXA-48-positive *Escherichia coli* and *Klebsiella pneumoniae*

Beatriz Gomes<sup>1</sup>, Vanessa Oliveira<sup>1</sup>, Elmano Ramalheira<sup>2</sup>, Sónia Mendo<sup>1</sup>, Sónia Ferreira<sup>1,3</sup>, Tânia Caetano<sup>1</sup>

<sup>1</sup>CESAM and Department of Biology, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

<sup>2</sup>Centro Hospitalar do Baixo-Vouga, EPE, Aveiro, Portugal

<sup>3</sup>Department of Medical Sciences, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

Email: [beatriz.a.gomes@ua.pt](mailto:beatriz.a.gomes@ua.pt)

With the development of several classes of antibiotics, infectious diseases were expected to be easily controlled<sup>1</sup>. However, this did not happen, and antibiotic resistance became an emerging problem. One of the most important resistance mechanisms used by bacteria is the production of enzymes capable of degrading antibiotics<sup>2</sup>. OXA-48 is a beta-lactamase, namely a carbapenemase, which degrades the beta-lactam ring present in the structure of beta-lactam antibiotics. The resistance conferred by this enzyme, which is mainly to carbapenems, is a matter of concern since these are known as “last resort antibiotics”<sup>3</sup>. This work aims to characterize, mostly at genetic level, OXA-48-positive *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from the same biological sample (pus). We aim to investigate the i) transmissibility potential of *bla*<sub>OXA-48</sub>, encoding the OXA-48, ii) identify the genetic environment of *bla*<sub>OXA-48</sub> and iii) identify virulence factors that may influence their pathogenicity. Extraction of total DNA showed that both strains have plasmids, where *bla*<sub>OXA-48</sub> is normally found. Thus, plasmid DNA was purified and used for transformation of *E. coli*. Transformants were obtained, exhibiting resistance to carbapenems, and *bla*<sub>OXA-48</sub> was successfully amplified by PCR from these strains. To verify the potential of horizontal gene transfer of *bla*<sub>OXA-48</sub>, conjugation assays were performed. Transconjugants harboring the *bla*<sub>OXA-48</sub> gene were obtained for both *E. coli* and *K. pneumoniae* and susceptibility testing confirmed their resistance towards carbapenems, showing that the plasmid *bla*<sub>OXA-48</sub> can be transferred and transcribed between different species. The *bla*<sub>OXA-48</sub> plasmid may have been transmitted from one species to the other *in vivo*, an event that is not uncommon and has been reported worldwide<sup>4,5</sup>. Genome sequencing results will allow to identify factors that may promote such biological behavior allowing to identify other characteristics that contribute in the infective potential, such as virulence factors.

### References

- <sup>1</sup>Saga T, Yamaguchi K. (2009), *Japan Medical Association Journal*, 52(2), 103-108.
- <sup>2</sup>Peterson E, Kaur . (2018), *Front Microbiology*, 9, 1-21.
- <sup>3</sup>Hirvonen VHA, Spencer J. (2021), *ASM Journals*, 65(6), 1-18.
- <sup>4</sup>Göttig S, Gruber TM, Stecher B, Wichelhaus TA, Kempf VAJ (2015), *Clinical Infectious Disease*, 60(12) 1808-1815.
- <sup>5</sup>Manageiro V, Ferreira E, Pinto M, Caniça M. (2014), *Antimicrob Agents Chemother*, 58(12), 7613-7614.

## Unveiling the genome of *Limtongozyma cylindracea*

Maria J Carvalho<sup>1</sup>, Miguel Pinheiro<sup>1</sup>, Andreia Reis<sup>1</sup>, Rita Bezerra<sup>1</sup>, Manuel AS Santos<sup>1,2</sup>, Gabriela Moura<sup>1</sup>

<sup>1</sup>Genome Medicine Laboratory, Institute of Biomedicine - iBiMED, Department of Medical Sciences, University of Aveiro, Portugal

<sup>2</sup>Multidisciplinary Institute of Ageing – MIA Portugal, Faculty of Medicine, University of Coimbra, Portugal

Email: [micarvalho@ua.pt](mailto:micarvalho@ua.pt)

*Limtongozyma cylindracea* (basionym: *Candida cylindracea*) is a biotechnologically long-used species especially due its low-specificity lipases extensively used for biodetergent production<sup>1</sup>. CUG (leucine) codons are 100% translated as serine by *L. cylindracea*, representing a crucial evolutionary point in yeasts phylogeny, where different clades translate this codon as Leu (standard decoders), Ala or Ser/Leu ambiguously<sup>2,3</sup>.

As eukaryotic unicellular organisms, yeasts present genomic characteristics that distinguish them from the most studied genomes (bacterial and human), rendering most open-source bioinformatics tools inappropriate for the study of fungal genomes. In the case of rare pathogens and rare organisms such as *L. cylindracea*, for which no genome sequence reference is available, the lack of bioinformatic tools further hinders the study of such organisms and, in the case of pathogens, resolving outbreaks. Using Illumina (Illumina) and Oxford Nanopore Technologies (ONT), we performed whole genome and RNA sequencing of *L. cylindracea* ATCC14830 and established a bioinformatics pipeline for *de novo* assembly and annotation of this genome.

Hybrid assembly using Flye(v2.9) and Pilon(v.1.23) resulted in 27 contigs (N50= 1.70Mbp; L50= 3 contigs), in a total of 10.28Mbp with nearly 63%GC. BUSCO(v4.1.4) and FGMP(v1.0.2) revealed 71.3% and 96.3% of genome completeness. For structural annotation, MAKER(v4.12.0) was used and 5132 genes obtained. Transcripts were annotated for their functional potential with InterProScan(v5.55-88.0) and 4816 proteins (94%) had at least one InterPro annotation, with 4048 proteins (79%) showing at least one Pfam. Gene Ontology (GO) analysis showed 5824, 4173, and 1651 GO annotations under biological process, molecular function, and cellular component, respectively. To identify putative virulence factors, we used the VFDF<sup>4</sup> database and obtained 1003 single matches. Additionally, 215 tRNA genes were predicted by tRNAscan-SE(v1.3.1). Further analyses are ongoing to unveil the evolutionary paths that allowed the complete reassignment of the CUG codon from Leu to Ser.

This work was financed through the project CANCYL-POCI-01-0145-FEDER-031849; GenomePT: POCI-01-0145-FEDER-022184; iBiMED: FCT UID/BIM/04501/2020.

### References

<sup>1</sup>Benjamin S, Pandey A. *Yeast*. 1998; 14(12):1069-87.

<sup>2</sup>Ueda T *et al.* *Biochimie*. 1994; 76(12):1217-22.

<sup>3</sup>Massey *et al.* *Genome Res*. 2003. 13: 544-557.

<sup>4</sup>Lu T, Yao B, Zhang C. DFVF: database of fungal virulence factors. Database (Oxford). 2012;2012:bas032. Published 2012 Oct 22. doi:10.1093/database/bas032.





SESSION 3  
EVOLUTIONARY GENETICS

## Demographic history using genomic data: can we separate fact from story?

Lounès Chikhi

CNRS Toulouse, France

IGC - Instituto Gulbenkian de Ciência, Oeiras, Portugal

Email: [chikhi@igc.gulbenkian.pt](mailto:chikhi@igc.gulbenkian.pt)

Genetic and genomic data are used to reconstruct the demographic history of populations. For instance, we may detect population bottlenecks by applying sophisticated methods to genomic data. By association with archeological or palaeontological data we may connect the "genetic bottleneck" to a putative history in which the real population size decreased. However, there is an increasing recognition that when populations are structured many methods ignoring population structure may detect spurious population size changes. In other words, a structured population with constant size or even increasing population size may actually exhibit a signal of bottleneck. In other words, genetic data may lead us to reconstruct a history that may never have taken place. During the talk I will present recent results related to our work and human evolution. In particular I will focus on the PSMC method of Li and Durbin and show how it can be used to reconstruct a history of structured populations with ancient changes in connectivity.

## Unraveling the genetic basis of drug resistance in *Candida albicans* strains with non-standard translation

Gonçalo Sousa<sup>1</sup>, Ana Rita Guimarães<sup>1</sup>, Miguel Pinheiro<sup>1</sup>, Carla Oliveira<sup>1</sup>, Gabriela Moura<sup>1</sup>, Manuel Santos<sup>1,2</sup>,  
Ana Rita Bezerra<sup>1</sup>

<sup>1</sup>Department of Medical Sciences, Institute of Biomedicine – iBiMED, University of Aveiro, Portugal

<sup>2</sup>Multidisciplinary Institute of Aging, MIA-Portugal, Faculty of Medicine, University of Coimbra, Portugal

Email: [armbezerra@ua.pt](mailto:armbezerra@ua.pt)

*Candida albicans* is the leading cause of life-threatening invasive infections with mortality rates approaching 40%, despite treatment. Resistance to the commonly used azoles is increasing and alternative antifungals, such as amphotericin B or echinocandins, increase the cost of antifungal therapy. Despite the economic and clinical relevance of antifungal drug resistance, this subject remains poorly studied in comparison with the similar issue of antibiotic resistance in bacterial pathogens. Amongst the interesting features of *C. albicans* is its ability to ambiguously translate the universal leucine CUG codon as serine (97%) and leucine (3%). Such non-standard translation produces a flexible proteome that generates phenotypic and genomic diversity. Here, we hypothesize that CUG codon ambiguity has an important role in the evolution of resistance to clinical antifungals. Using strains with variable CUG-Leu misincorporation rates, we combined experimental evolution and whole-genome sequencing to elucidate evolutionary paths leading to the emergence of resistance to two major classes of antifungals (polyenes, azoles). Results showed that high levels of mistranslation accelerate the acquisition of azole resistance, but not polyene resistance. Hyper mistranslation caused more rapid and frequent evolution of fluconazole resistance mediated through CNVs affecting the classical drug efflux and ergosterol biosynthesis pathways, while itraconazole resistant isolates showed aneuploidies affecting transport. In the evolution with the polyene Amphotericin B, hyper mistranslation had little impact in the frequency of resistance acquisition and most of the genome changes detected were SNPs and INDELS in filamentation genes.

This work is supported by the Portuguese Foundation for Science and Technology (FCT), POCI- COMPETE2020 and FEDER through grant PTDC/BIA-MIC/1141/2021. The iBiMED research unit is supported by FCT funds under UIDP/04501/2020.

## Genomic evolution during experimental adaptation of life histories and aging in *Caenorhabditis elegans* with different microbes

Josiane Santos<sup>1</sup>, David Pires<sup>1,2</sup>, Anke Konrad<sup>1</sup>, Ivo M Chelo<sup>1</sup>

<sup>1</sup>cE3c – Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências,  
Universidade de Lisboa, Lisboa, Portugal

<sup>2</sup>INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Av. da República,  
Quinta do Marquês, Oeiras

Email: [immchelo@fc.ul.pt](mailto:immchelo@fc.ul.pt)

Host-microbe interactions are a major environmental context for healthy aging of the host and likely mediate trade-offs between life-history traits in the evolution of host senescence. Here we have used experimental evolution of the nematode *Caenorhabditis elegans* to examine how host-microbe interactions determine the evolution of host life-history and aging. We start by showing that there is a substantial, host-genotype-dependent impact on reproductive effort and survival of the nematode host and that two non-pathogenic *Escherichia coli* strains, a pathogenic *E. coli* strain and a pathogenic *Serratia marcescens* strain impose specific environments where unique evolution of life-histories can take place. Genome-wide polymorphisms obtained from experimental populations evolved for 50 generations under selection for late-reproduction provide a first glimpse on the genetic architecture of aging evolution and its dependence on the interaction between immunity, development and cell metabolism.

## Adaptive genomics and ecological resilience: biological insights

Agostinho Antunes<sup>1, 2</sup>

<sup>1</sup>CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208 Porto, Portugal

<sup>2</sup>Department of Biology, Faculty of Sciences, University of Porto. Rua do Campo Alegre, 4169-007 Porto, Portugal

Email: [aantunes@ciimar.up.pt](mailto:aantunes@ciimar.up.pt)

Two decades following the completion of the human genome sequencing in 2001, provided the means and methods for extend the importance of whole genome sequencing projects to a wide range of taxa, with thousands of species being the target of whole genome sequencing, from simple bacteria to more complex organisms, such as mammals. Such voluminous sequencing data generated across multiple phylogenetically diversified organisms provides also the framework to better understand the genetic makeup of such species and related ones, allowing to explore the genetic changes underlining the evolution of diverse phenotypic and adaptive traits and their relevance for conservation. Here, recent results from our group retrieved from comparative evolutionary genomic analyses of varied endangered metazoan species will be considered to exemplify how gene novelty and gene enhancement by positive selection might have been determinant in the success of adaptive radiations into diverse environments and lifestyles. The findings pinpoint unique molecular products of critical relevance in species evolution, diversification, ecological resilience and conservation, but further highlight genomic novelties relevant for environmental and biomedical research.

## A phylogenetic and evolutionary analysis of transposable elements in the wolf (*Canis lupus*, *Canidae*, *Carnivora*) genome

Maria Gaspar<sup>1</sup>, Raquel Chaves<sup>1,2</sup>, Maris Hindrikson<sup>3</sup>, Jorge Pereira<sup>4</sup>, Filomena Adegas<sup>1,2</sup>

<sup>1</sup>Laboratory of Cytogenomics and Animal Genomics, DGB - Department of Genetics and Biotechnology, UTAD - University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>2</sup>BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

<sup>3</sup>Department of Zoology, University of Tartu, Estonia

<sup>4</sup>Animal and Veterinary Research Centre (CECAV), AL 4AnimalS, UTAD – University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Email: [mariatxi.gaspar@gmail.com](mailto:mariatxi.gaspar@gmail.com)

Transposable Elements (TEs) are DNA sequences that can move within the species genome and influence the appearance, regulation, and evolution of genomes. One of these elements, Long Interspersed Nuclear Element 1 (LINE-1) is highly present in most mammal genomes, but there aren't studies on the *Canis* genome.

We used two cell lines (male and female) from European Wolf (*Canis lupus lupus*) stipulated in our lab. Cytogenetics characterization through G- and C-banding revealed a karyotype of 78 chromosomes, with 38 pairs of acrocentric autosomes and 2 sex chromosomes, with a submetacentric X and acrocentric Y, identified by chromosome painting. *Canis lupus* is accepted as the *Canis lupus familiaris* (dog) ancestor that also harbours 78 chromosomes, but little is known about the differences of the two related genomes.

LINE-1 elements were isolated from genomic DNA of wolf using specific primers and used as probes in FISH (Fluorescence *in Situ* Hybridization) to physically map these TEs in wolf chromosomes. This analysis was extended to the dog, allowing a qualitative and semi quantitative analysis and comparison between these two phylogenetically related genomes. An interspersed pattern distribution throughout the chromosomes was observed with an apparent higher LINE-1 content in the wolf genome.

An in depth *in silico* analysis was performed on available Genome project sequences from both species, allowing to search for the presence and exact location of LINE-1 in the chromosomes and perform a phylogenetic analysis on the sequences intra and inter genomes. A combined analysis of the *in silico* and cellular analysis of LINE-1 and the syntenies' analysis of the dog chromosomes with the reference genome of *Carnivora (Felis catus)* allowed to disclose the possible involvement of these TEs in the evolutionary pathway of the dog genome, and allowed, as well, to extrapolate it to the wolf genome for the first time.

## **Beyond “old genes, new tricks”: the genes behind the development of novel traits**

Patrícia Beldade, Roberto Arbore, Carolina Peralta, Suzanne Saenko

Faculty of Sciences, University of Lisbon

cE3c: Centre for Ecology, Evolution, and Environmental Changes

DBA: Department of Animal Biology

Evolutionary novelties, or traits that are restricted, and often characteristic, of specific lineages, are among the most fascinating examples of phenotypic diversification. Examples of such traits include flowers in angiosperms, feathers in birds, placenta in mammals and scale-based color patterns on lepidopteran wings. Studies in different taxa and different traits have shown that the genes behind the development of novel traits are themselves not novel. Rather, there is substantial evidence that the evolutionary origin of novel traits relies on the co-option of shared, ancestral genetic circuitry, which acquires additional and novel functions in particular lineages. This has been summarized in the expression “old genes, new tricks”. I will present work on the identification and functional characterization of genes involved in the formation of butterfly wing patterns which challenge this idea in different ways. First, I will discuss work showing how the knowledge of the “old genes’ old functions” can mislead the quest to identify the genetic basis of variation in novel traits. And second, I will discuss work showing the involvement of a lineage-restricted gene on the formation of a lineage-restricted trait.

## Lepidopteran prolegs are novel traits not thoracic leg serial homologs

Yuji Matsuoka<sup>1</sup>, Suriya Murugesan<sup>1</sup>, Anupama Prakash<sup>1</sup>, and Antónia Monteiro<sup>1</sup>

<sup>1</sup>National University of Singapore

Email: [antonia.monteiro@nus.edu.sg](mailto:antonia.monteiro@nus.edu.sg)

Lepidopteran larvae have both thoracic legs and abdominal prolegs, yet it is unclear whether these legs are homologous. We examined the role of three Hox genes in proleg development in *Bicyclus anynana* butterflies using CRISPR-Cas9 and discovered that under a partial segment *abdominal-A* (*abd-A*) knockout, both types of leg can develop in the same segment, arguing for prolegs being a novel trait, not a leg serial homolog. We also discover that *specificity protein* (*sp*) genes do not co-localize with Distal-less (Dll) in prolegs, as they do in legs, and that the proleg gene-regulatory network (GRN) mostly resembles the head-horn GRN, another novel trait in the lepidoptera. We propose that larval prolegs evolved from the co-option of a partial limb GRN into novel embryonic coordinates in the abdomen.



## Peeking into Daubenton's bat comparative chromosome map- A preliminary study

Verónica F. Mestre<sup>1,2,3</sup>, Jorge Pereira<sup>4</sup>, Paulo Barros<sup>3</sup>, Sandra Faria<sup>3</sup>, Raquel Chaves<sup>1,2</sup>, David Ray<sup>5</sup>, João A. Cabral<sup>3</sup>, Filomena Adegas<sup>1,2</sup>

<sup>1</sup>Laboratory of Cytogenomics and Animal Genomics, DGB - Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>2</sup>BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

<sup>3</sup>Laboratory of Applied Ecology, Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>4</sup>Animal and Veterinary Research Centre (CECAV), AL 4AnimalS, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>5</sup>Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA

Email: [vero.mestre11@gmail.com](mailto:vero.mestre11@gmail.com)

The family *Vespertilionidae* is the largest family of the Chiroptera order, and is highly distributed among the world. Within the Vesper bats, the mouse eared bats (*Myotis*) exhibit a very conserved karyotype, with  $2n=44$  chromosomes<sup>1</sup>, with only a few exceptions. Through cytogenetics approaches recurring to Zoo-FISH methodologies it is possible to compare the genomes of very different species<sup>2</sup>, what gives us hints on the possible karyotype rearrangements occurred throughout evolution. In this work we present a partial comparative chromosome map of *Myotis daubentonii* using human painting probes. Moreover, we are doing an integrated *in silico* analysis using the chromosome maps of *Myotis myotis* in comparison to the human karyotype<sup>3</sup>. This comparison will allow us to disclose the extent and type of rearrangements occurred between these *Myotis* species and between these and human. Furthermore, this work can shed the light on the evolutionary events that shaped these extant species genomes, as well this analysis will also give us the genome coordinates of evolutionary breakpoints, and consequently the sequences lying on those regions, that most probably contributed to karyotype evolution.

### References

<sup>1</sup>Volleth, M., & Heller, K. G. (2012). *Vespertilio*, 16, 329-350

<sup>2</sup>O'Brien, S. J., Menotti-Ramond, M., Murphy, W. J. et al. (1999) *Science*, 286, 458-480

<sup>3</sup>Volleth, M., Heller, K. G., Pfeiffer, R. A., & Hameister, H. (2002). *Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*, 10(6), 477-497

## The genetic basis of bacterial adaptation to a complex environment and its consequences on predator growth

Ana Paula Marques<sup>1,2</sup>, [Ivo M Chelo](#)<sup>1</sup>

<sup>1</sup>CE3c – Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

<sup>2</sup>iBET – Instituto de Biologia Experimental e Tecnológica, Av. República, Qta. do Marquês Edifício iBET/ITQB, Oeiras-Portugal

Email: [immchelo@fc.ul.pt](mailto:immchelo@fc.ul.pt)

Under the most simple expectations, adaptation of a microbial species to changing abiotic conditions should benefit other species that are affected positively by it, such as in the case of mutualisms or consumer/resource type interactions. This is particularly relevant given how bacteria can affect other organisms' life-history parameters and their ability to adapt to stressful conditions. In this work, we focus on a predator/prey experimental system with *Escherichia coli* and *Caenorhabditis elegans* to explain how adaptation of one species to a novel environment determines adaptation of the other species to the same novel abiotic environment. Twenty independent lines of *E. coli* OP50 strain were maintained for ~ 1000 generations in solid NGM (nematode growth medium), at 20 °C, which characterizes commonly used conditions for *C. elegans* growth. We describe the genetic basis of microbial adaptation to this complex environment and how this affects *C. elegans* (predator) performance. Despite a great variety of genes targeted during adaptation we find evidences of parallel evolution associated with the master flagellar operon *flhDC*, which is surprisingly unrelated with motility. Furthermore, we also found that evolved *E. coli* populations can have marked effects on the nematode's ability to grow. By comparing results with those from a replicated experiment under high salt concentration we test our original hypothesis that an improved microbial metabolism allows *C. elegans* to cope better with stressful environments. Our data indicates that adapted *E. coli* are in general more detrimental to *C. elegans* than their ancestors, which results from an underlying antagonistic interaction that can be put to evidence by an imposed delay in the nematode's developmental rate.

## Mitochondrial DNA characterization of Brazilian immigrant population living in Lisboa

Miguel Marcelino<sup>1,2</sup>, António Amorim<sup>1,2</sup>, Francisco Corte Real<sup>3,4</sup>, Heloísa Afonso Costa<sup>1</sup>

<sup>1</sup>Serviço de Genética e Biologia Forenses, Delegação do Sul do Instituto Nacional de Medicina Legal e Ciências Forenses, I.P, Lisboa

<sup>2</sup>Faculdade de Ciências da Universidade de Lisboa, Lisboa

<sup>3</sup>Instituto Nacional de Medicina Legal e Ciências Forenses, I.P, Coimbra

<sup>4</sup>Faculdade de Medicina da Universidade de Coimbra, Coimbra

Email: [fc55868@alunos.fc.ul.pt](mailto:fc55868@alunos.fc.ul.pt)

Migration is one of the main factors for genetic variability within populations. Currently, the Portuguese population, and particularly the population from Lisboa, welcomes a considerable number of immigrants. Brazilian immigrants are the main foreign community in Portugal, with about 184 000 individuals in 2020.

Mitochondrial DNA (mtDNA), due to its unique characteristics such as being exclusively maternal inheritance and suffering no recombination, which results in its slow evolution, is a useful genetic marker to study the evolution of populations.

In this study mtDNA sequencing analysis of 64 Brazilian immigrants who currently live in Lisbon were carried out in order to assess the impact of this population on the Portuguese gene pool.

The mtDNA control region were amplified using two pairs of primers - L15971 / H016 and L16555 / H639. The amplified products were then sequenced using BigDye<sup>®</sup> Terminator v.3.1 Cycle Sequence (AB) and detected in the SeqStudio<sup>™</sup> Genetic Analyzer (AB). The results were analysed with the Sequencing Analysis v7. and SeqScape v4. (AB) softwares, where the obtained sequences were compared with the rCRS in order to obtain haplotypes that, with Phylotree, build 17, can be converted in haplogroups.

From our results, it is possible to confirm that this Brazilian population living in Lisboa presents a high number of unique haplotypes, most of them had no coincidence on EMPOP Forensic data. It's an extremely heterogeneous population with half of the studied haplotypes belonging to macrohaplogroup L, characteristic mainly of Sub-Saharan region of Africa, and a quarter of the studied haplotypes belonging to South American population and the other quarter belonging to Euro Asiatic population



## The genome landscape of native Iberian cattle

Catarina Ginja

BIOPOLIS-CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Portugal

Email: [catarinaginja@cibio.up.pt](mailto:catarinaginja@cibio.up.pt)

Iberian native cattle breeds exhibit a remarkable phenotypic diversity over a limited geographical space. These cattle are found in diverse agro-ecological systems including coastal, mountain, and lowland arid environments, and their inheritable traits have been modified at different times by the various cultures that inhabited this territory. The complex origin of these breeds is reflected in their high diversity in Y-chromosome haplotypes, including the major taurine Y1 and Y2 haplogroups<sup>1,2</sup> and unique patriline<sup>3</sup>, as well as distinct maternal lineages, i.e. common European T3-matrilines along with more distinct Q-haplotypes<sup>2</sup>, and a strong influence of T1-lineages of African origin<sup>4</sup>. Indeed, the genetic legacy of Iberian breeds is still represented in Creole cattle from the Americas as shown by classic genetic markers<sup>5</sup>. The patterns of genomic diversity observed in Iberian native breeds will be discussed in the context of worldwide cattle using high-throughput sequence data. Questions such as to which extent native cattle from this territory represent relics from the past will also be addressed considering Archaeogenomic results obtained for specimens collected in the Iberian Peninsula.

### References

- <sup>1</sup>Edwards CJ, Ginja C, Kantanen J, Pérez-Pardal L, et al. (2013). *PLoS One*, 6, e15922. <https://doi.org/10.1371/journal.pone.0015922>.
- <sup>2</sup>Ginja C, Penedo MCT, Melucci L, Quiroz J, et al. (2010). *Animal Genetics*, 41, 128–41. <https://doi.org/10.1111/j.1365-2052.2009.01976.x>. [cited 2013 Oct 25].
- <sup>3</sup>Pelayo R, Penedo MCT, Valera M, Molina A, et al. (2017). *Animal Genetics*, 48, 450–4. <https://doi.org/10.1111/age.12549>. [cited 2018 Jul 6].
- <sup>4</sup>Bonfiglio S, Ginja C, De Gaetano A, Achilli A, et al. (2012). *PLoS One*, 7, e38601. <https://doi.org/10.1371/journal.pone.0038601>.
- <sup>5</sup>Ginja C, Gama LT, Cortés O, Martin-Burriel I, et al. (2019). *Scientific Reports*, 9, 11486. <https://doi.org/10.1038/s41598-019-47636-0>

## **Bailundo pigs: whole genome sequencing shows unique regions of selection for metabolism and feed efficiency**

Pedro Sá<sup>1,2</sup>, Dulce Santos<sup>1,2</sup>, Hermenegildo Chiaia<sup>3</sup>, Alexandre Leitão<sup>1,2</sup>, José Moras Cordeiro<sup>3</sup>, Luís Telo da Gama<sup>1,2</sup>, Andreia Jesus Amaral<sup>1,2</sup>

<sup>1</sup>CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>2</sup>Laboratório Associado para a Ciência Animal e Veterinária (AI4Animals), Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>3</sup>Faculdade de Medicina Veterinária, Universidade José Eduardo dos Santos, Huambo, Angola

Email: [andreiaamaral@fmv.ulisboa.pt](mailto:andreiaamaral@fmv.ulisboa.pt)

Angola has been through dramatic social events that have led to the disappearance of indigenous swine populations, which are also threatened by the recent introduction of European exotic breeds. In an effort to investigate the genetic basis of remnant indigenous pig populations in Angola, we have studied a local extant population from *Bailundo*, using whole genome sequencing. The results were compared with 78 genomes of European and Asian domestic pig breeds as well as European and Asian wild boars, which information is currently in the public domain. The analyses of population structure showed that *Bailundo* pigs have a closer relatedness with European pig breeds and are very distant from Asian pig breeds. Pairwise  $F_{ST}$  for the various breeds ranged from 0.14 to 0.26, and *Bailundo* pigs display lower levels of genetic differentiation relative to European breeds. Finally, we have identified candidate regions involved in selection, using a complementary approach based on the use of three different methods that allow the identification of selection footprints that have emerged in different timescales. Candidate genes identified as being under selection in *Bailundo* pigs were mostly involved in biological processes related with feed efficiency and metabolism. This study presents the first assessment of the genetic relationship between an indigenous pig population from Angola and worldwide pigs, and shows that those pigs harbour unique genetic attributes that should be further explored and conserved.

## Cytogenetic Analysis of N-Methyl-N-Nitrosourea (MNU) Induced Mammary Tumour in Female Wistar Rat

Abigaël Valada <sup>1</sup>, Ana- I. Faustino Rocha<sup>1,2</sup>, Maria Miranda<sup>1</sup>, Jessica Silva<sup>1</sup>, Tiago Azevedo<sup>1</sup>, Manuela Matos<sup>3</sup>,  
Rosário Pinto Leite<sup>4</sup>, Paula Oliveira<sup>1,5</sup>

<sup>1</sup>CITAB- Ino4gro, UTAD Vila Real, Portugal

<sup>2</sup>Comprehensive Health Research Center, Évora, Portugal

<sup>3</sup>Department of Genetic and Biotechnology, UTAD, Vila Real, Portugal

<sup>4</sup>Genetics Laboratory of the Trás-os-Montes e Alto Douro Hospital Centre, Vila Real, Portugal

<sup>5</sup>Department of Veterinary Sciences, CECAV, UTAD, Vila Real, Portugal

Email: [anafaustino@uevora.pt](mailto:anafaustino@uevora.pt)

Breast cancer is the most common cancer diagnosed in women, accounting for more than 1 in 10 new cancer diagnoses each year<sup>1</sup>. Animal models are widely used to study the mechanisms of mammary carcinogenesis. N-Methyl-N-Nitrosourea (MNU) is a DNA-interacting alkylating agent that has been used extensively in rats to induce mammary cancer (adenocarcinoma)<sup>2</sup>. However, the cytogenetic characterization of rat mammary tumours is scarce. The aim of this study was to perform a cytogenetic analysis of the MNU-induced mammary tumors in female Wistar rats.

Five tumours samples were collected, in sterile conditions, from 5 female Wistar Uniliver rats that have been exposed, at 50 days of age, to an intraperitoneal injection, with N-Methyl-N-Nitrosourea (MNU) during 18 weeks. All cell culture and manipulation processes until obtaining the metaphases and banding were performed according to the protocols established in the laboratory. A total of 400 metaphases (80 per tumor) were analyzed and in all tumors a normal rat karyotype (n=42) was observed. The percentage of aneuploidy (considered in a range of 36 to 48 chromosomes) detected varies between tumours: 48.75% in tumor two, 55% in both tumours four and five, 96.25% in tumor three and 98.75% in tumor one. In addition, the presence of endoreplications were observed in tumor one (n=5), tumor four (n=13) and tumor five (n=9). With this preliminary study, we have observed that MNU methylation may have induced aneuploidies in the five mammary tumors investigated. When extrapolated to humans, these abnormalities could potentially correspond to early cytogenetic abnormalities in mammary cancer. More studies by molecular cytogenetics and genetics need to be performed, in order to detect some structural rearrangements and genetic polymorphism to better characterize these tumors.

### References

- <sup>1</sup>Alkabban FM, Ferguson T. Breast Cancer. 2021 Aug 7. In: *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan*– PMID: 29493913.
- <sup>2</sup>Faustino-Rocha, A. I., Ferreira, R., Oliveira, P. A., Gama, A., & Ginja, M. (2015). N-Methyl-N-nitrosourea as a mammary carcinogenic agent. *Tumor Biology*, 36(12), 9095–9117.

## Analysis of sequence variability of prion protein gene (*PRNP*) in Portuguese cervid populations species: Red deer, Fallow deer and Roe deer

Jorge C. Pereira<sup>2,3</sup>, Nuno Gonçalves-Anjo<sup>1,4</sup>, Estela Bastos<sup>1,4</sup>, Leonor Orge<sup>3,6</sup>, Ana C. Matos<sup>5</sup>, Adelina Gama<sup>3</sup>, Anabela Alves<sup>3</sup>, Alexandra Esteves<sup>3</sup>, Sara Rocha<sup>1</sup>, Luís Figueira<sup>5</sup>, Carla Lima<sup>6</sup>, Filipe Silva<sup>3</sup>, Fernanda Seixas<sup>3</sup>, Isabel Pires<sup>3</sup>, João Silva<sup>6</sup>, Madalena Vieira-Pinto<sup>3</sup>, Maria L. Pinto<sup>3</sup>, Paula Mendonça<sup>6</sup>, Paulo Carvalho<sup>6</sup>, Paula Tavares<sup>6</sup>, Roberto Sargo<sup>3</sup>, Maria A. Pires<sup>3</sup>

<sup>1</sup>Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>2</sup>Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>3</sup>Veterinary and Animal Science Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>4</sup>CITAB Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>5</sup>Polytechnic Institute of Castelo Branco (IPCB), 6000-084, Castelo Branco, Portugal

<sup>6</sup>Pathology Laboratory, UEISPSA, National Institute for Agricultural and Veterinary Research (INIAV), I.P., Oeiras and Vairão, Portugal

Email: [jcpereira@utad.pt](mailto:jcpereira@utad.pt)

Transmissible Spongiform Encephalopathy (TSE) or prion diseases are a family of neurodegenerative diseases caused by lethal infectious pathogens called Prions. Among the transmissible spongiform encephalopathies (TSEs), chronic wasting disease (CWD) in cervids is now the rising concern in wildlife within Europe, after the first case was detected in Norway in 2016, in a wild reindeer and until October 2021, a total of 40 cases were described in Norway, Sweden and Finland. The study of the genetics of the prion protein gene, *PRNP*, has been proved to be a valuable tool for determining the relative susceptibility to TSEs. In the present study we analyzed the exon 3 of *PRNP* gene in 235 samples from three Portuguese cervid species: red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and Roe deer (*Capreolus capreolus*). One synonymous – codon A136A – and two non-synonymous variations - codon T98A, codon Q226E - were found in red deer while no sequence variation was found in fallow deer and roe deer. All animals in the present study, were previously screened and were negative for the presence of CWD. The comparison of our population with North American populations, suggest that the free-ranging deer from our study may present susceptibility to CWD, although lack of experimental data and the necessity of large survey are necessary to evaluate these populations. The establishment of risk assessment projects, even in countries with no cases of CWD is very important to forecast possible contaminations.

This work was supported by the projects Project 029947IC&T 02/SAICT/2017-SAICT funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/04033/2020 funded by the Portuguese Foundation for Science and Technology (FCT).



## Are Western Iberian Roe deer populations hotspots of genetic diversity?

Jorge C. Pereira<sup>2,3</sup>, A. Machado<sup>1</sup>, Mafalda Saianda<sup>1</sup>, Nuno Gonçalves-Anjo<sup>1,4</sup>, Estela Bastos<sup>1,4</sup>, Leonor Orge<sup>3,6</sup>, Ana C. Matos<sup>5</sup>, Adelina Gama<sup>3</sup>, Anabela Alves<sup>3</sup>, Alexandra Esteves<sup>3</sup>, Sara Rocha<sup>1</sup>, Luís Figueira<sup>5</sup>, Carla Lima<sup>6</sup>, Filipe Silva<sup>3</sup>, Fernanda Seixas<sup>3</sup>, Isabel Pires<sup>3</sup>, João Silva<sup>6</sup>, Madalena Vieira-Pinto<sup>3</sup>, Maria L. Pinto<sup>3</sup>, Paula Mendonça<sup>6</sup>, Paulo Carvalho<sup>6</sup>, Paula Tavares<sup>6</sup>, Roberto Sargo<sup>3</sup>, Maria A. Pires<sup>3</sup>

<sup>1</sup>Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>2</sup>Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>3</sup>Veterinary and Animal Science Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>4</sup>CITAB Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (UTAD), 5000-801, Vila Real, Portugal

<sup>5</sup>Polytechnic Institute of Castelo Branco (IPCB), 6000-084, Castelo Branco, Portugal

<sup>6</sup>Pathology Laboratory, UEISPSA, National Institute for Agricultural and Veterinary Research (INIAV), I.P., Oeiras and Vairão, Portugal

Email: [jcpereira@utad.pt](mailto:jcpereira@utad.pt)

Given its geographic isolation, and after the last glaciation, the Iberian Peninsula, become a sanctuary of plants and animal's diversification, being considered a hotspot of genetic diversity specially for mammal species. The roe deer (*Capreolus capreolus* - CCR), an herbivore ungulate, has a widespread geographical distribution which extends from the Iberian Peninsula to the north of Scandinavia, being found as well in Turkey, Israel, and Jordan. *C. capreolus* populations have been subject to a significant number of fluctuations, especially due to anthropogenic activities, which led to a decrease in size and distribution. These alterations combined with translocations of animals of the same species, can lead to meaningful consequences on the genetic structure, diversity, and fitness of populations.

In this study we aimed to outline the genetic diversity and structure of Western Iberian roe deer populations using mitochondrial DNA (mtDNA) molecular analysis. Previous studies in Western Iberian roe deer populations using this genetic marker, showed a high degree of genetic diversity, with shared gene pools with other European and Iberian regions, but also with unique genetic elements. Here we present preliminary results from the analysis of 65 roe deer samples from different Western Iberian areas (wild and fenced populations). From 60 samples it was possible to amplify the mtDNA fragment of interest, with a length of 436 bp. The sequencing analysis of 30 of these 60 samples revealed the presence of 13 haplotypes already identified in other populations of roe deer.

These studies are of great importance to obtain information about the phylogeography, which can be used to design appropriate strategies for the conservation and management of populations, but as well to maintain the genetic heritage of roe deer in Europe.

This work was supported by the projects Project 029947IC&T 02/SAICT/2017-SAICT funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/04033/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

## Can HSAT1 colocalize with hotspots for chromosomal breaks?

Gabriela Veríssimo<sup>1,2</sup>, Mariana Lopes<sup>1,2</sup>, Marisa Fonseca-Carvalho<sup>1,2</sup>, Daniela Ferreira<sup>1,2</sup>, Sandra Louzada<sup>1,2</sup>, Raquel Chaves<sup>1,2</sup>

<sup>1</sup>Laboratory of Cytogenomics and Animal Genomics (CAG), Department of Genetics and Biotechnology (DGB); University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>2</sup>Biosystems and Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal

Email: [slouzada@utad.pt](mailto:slouzada@utad.pt)

The tandemly repeated satellite DNAs (satDNA) are an important organizational component of the human genome that can be found in heterochromatin-rich chromosomal regions, being the largest arrays organized within centromeric and pericentromeric regions<sup>1</sup>. Satellite DNA can vary widely in the human population in copy number and repeat structure<sup>2</sup>. Also, they have been associated with the occurrence of chromosomal rearrangements both in evolutionary and disease context by co-localizing with hotspots of recurrent breakpoint regions<sup>3</sup>.

The classical satellite DNA 1 (HSAT1) constitutes the most AT-rich portion of the human genome<sup>4</sup>. Initial studies cytogenetically mapped HSAT1 in the short arm of human acrocentric chromosomes, although this sequence has been systematically underrepresented in the reference genome. A recent effort to complete the remaining gaps on the human genome brought HSAT1 into light by showing its overall localization and organization in the human chromosomes<sup>5</sup>. Although, HSAT1 remains one of the poorly characterized human satDNAs.

Here we present preliminary results of a study aiming to characterize HSAT1 abundance and physical location in the genome of several human tumor and non-tumor cell lines. Absolute monomer copy number of HSAT1 was determined by qPCR and physical mapping was performed by fluorescence *in situ* hybridization (FISH). Our analysis shows the co-localization of HSAT1 with chromosomal rearrangement breakpoint regions. As this co-localization suggests that HSAT1 may be a genomic region more prone for the occurrence of breaks we next performed comet-FISH to assess if the DNA breaks occurred within this sequence. Our preliminary results suggest a possible association of HSAT1 to the occurrence of chromosomal rearrangements, indicating that this sequence may have a relevant role in chromosomal instability.

This work was supported by the Scientific Employment Stimulus (CEECIND/01825/2017), the project EXPL/BIA-OUT/1028/2021 and the Ph.D. grant (SFRH/BD/147488/2019), all from the Science and Technology Foundation (FCT) from Portugal.

### References

- <sup>1</sup>Plohl M, Luchetti A, Mestrovic N, & Mantovani B. (2008). *Gene*, 409(1-2), 72–82.
- <sup>2</sup>Miga KH (2019). *Genes (Basel)*, 10(5), 352.
- <sup>3</sup>Louzada S, Lopes M, Ferreira D, Adegas F, Escudeiro A, Gama-Carvalho M, Chaves R. (2020) *Genes (Basel)*, 11(1), 72.
- <sup>4</sup>Lopes, M., Louzada, S., Gama-Carvalho, M., & Chaves, R. (2021). *International journal of molecular sciences*, 22(9), 4707.
- <sup>5</sup>Nurk, S., Koren, S., Rhie, A., Rautiainen, M., Bizkadez, A. V., Mikheenko, A., Vollger, M. R., Altemose, N., Uralsky, L., Gershman, A., Aganezov, S., Hoyt, S. J., Diekhans, M., Logsdon, G. A., Alonge, M., Antonarakis, S. E., Borchers, M., Bouffard, G. G., Brooks, S. Y., Caldas, G. V., ... Phillippy, A. M. (2022). *Science (New York, N.Y.)*, 376(6588), 44–53.

## ***deltaC* 3'UTR is essential for zebrafish Embryo Clock oscillations**

Gil Carraco<sup>1,2,3</sup>, Alene Tavares<sup>1,2</sup>, Lara Carvalho<sup>4</sup>, Leonor Saúde<sup>4</sup>, Marco A. Campinho<sup>1,2</sup>, Alexander F. Schier<sup>3,5</sup>, Raquel P. Andrade<sup>1,2,6</sup>

<sup>1</sup>ABC-RI, Algarve Biomedical Center Research Institute, Faro, Portugal,

<sup>2</sup>Faculdade de Medicina e Ciências Biomédicas (FMCB), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>3</sup>Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA

<sup>4</sup>Instituto de Medicina Molecular - João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

<sup>5</sup>Biozentrum, University of Basel, Basel, Switzerland

<sup>6</sup>Champalimaud Research Program, Champalimaud Center for the Unknown, Lisbon, Portugal.

Email: [gdcarraco@ualg.pt](mailto:gdcarraco@ualg.pt)

Vertebrate embryo body segmentation is a time-controlled process. It takes approximately 30 minutes to form one pair of somites, the precursor structures of the axial skeleton, in zebrafish<sup>1</sup> and 4-5 hours in humans<sup>2</sup>. Accompanying somitogenesis, and with the same periodicity, there is a molecular mechanism characterized by oscillations of gene expression called the Embryonic Clock (EC). Mutations in EC genes lead to severe defects in axial skeleton development, and underly Human congenital diseases such as Spondylocostal Dysostosis<sup>3</sup>.

Knowing that a fast mRNA decay contributes to the proper functioning of the EC<sup>4</sup>, we performed a series of deletions in the 3'UTR of *deltaC*, a zebrafish core EC gene, using CRISPR-cas9 technology. We observed perturbations to the EC when we removed the medial 3'UTR ( $\Delta$ RR2 mutant) or the most distal part of the 3'UTR ( $\Delta$ RR3 mutant). Significant stabilization of the *deltaC* mRNA was observed in the  $\Delta$ RR3 mutant. Moreover, oscillatory behavior of *her7*, another EC core gene, was impaired in both mutants. Importantly, somitogenesis was perturbed in both mutants, having formed fewer and smaller somites than the wildtype counterparts and we found that this phenocopied the overactivation of the Notch signaling pathway.

This work demonstrates the relevance of the 3'UTR regulatory region of *deltaC* for proper EC operation, as alterations in this structure led to an impairment of gene oscillations, consequently promoting segmentation abnormalities.

### **References**

<sup>1</sup>Kimmel, C. B., W. W. Ballard, S. R. Kimmel, B. Ullmann and T. F. Schilling (1995). *Dyn*, 203(3), 253-310.

<sup>2</sup>Muller, F. and R. O'Rahilly (1986). *Am J Anat* 177(1), 3-19.

<sup>3</sup>Nobrega, A., A. C. Maia-Fernandes and R. P. Andrade (2021). "Altered Cogs of the Clock: Insights into the Embryonic Etiology of Spondylocostal Dysostosis." *J Dev Biol* 9(1).

<sup>4</sup>Hilgers, V., O. Pourquié and J. Dubrulle (2005). *Developmental Biology* 284(2), 292-300.

## Histopathological and cytogenetic characterization of canine mammary tumours

Maria Miranda<sup>1\*</sup>, Tiago Ferreira<sup>1\*</sup>, Ana I. Faustino-Rocha<sup>1,2</sup>, Hugo Brancal<sup>3</sup>, Fernando Leal<sup>4</sup>, Luís Carvalho<sup>5</sup>, José Chaves<sup>6</sup>, Adelina Gama<sup>7</sup>, Paula A. Oliveira<sup>1</sup>, Rosário Pinto-Leite<sup>8</sup>

<sup>1</sup>CITAB, Inov4Agro, UTAD, Vila Real, Portugal;

<sup>2</sup>Comprehensive Health Research Center, Évora, Portugal;

<sup>3</sup>Clínica Veterinária da Covilhã, Boidobra, Covilhã, Portugal;

<sup>4</sup>Clínica Veterinária de Felgueiras, Felgueiras, Portugal;

<sup>5</sup>Hospital Veterinário da Marinha Grande, Marinha Grande, Portugal;

<sup>6</sup>Marão Vet, Vila Real, Portugal;

<sup>7</sup>Animal and Veterinary Research Center (CECAV), UTAD, Vila Real, Portugal.

<sup>8</sup>Genetics/Andrology Laboratory, Centro Hospitalar de Trás-os-Montes e Alto Douro (CHTMAD), Vila Real, Portugal.

\*Both authors contributed equally to this work

Email: [anafaustino@uevora.pt](mailto:anafaustino@uevora.pt)

Canine mammary tumours (CMTs) show relatively high incidence; however, cytogenetic data are scarce, and little is known about chromosomal aberrations in these tumours<sup>1,2</sup>. This work presents a histological and cytogenetic analysis of CMTs. Eleven spontaneous CMTs were surgically excised and submitted for histopathological examination after fixation in 10% neutral buffered formalin. Before surgery, one blood sample was collected for peripheral blood culture. Primary cell cultures were established from fresh tumour tissues and cytogenetic processing, conventional GTL- and CGB-banding, were performed. In terms of histology, mammary lesions were classified as carcinomas: five (45.46%) papillary/tubulopapillary carcinomas, five (45.46%) non-simple malignant carcinomas (complex/mixed carcinomas) and one (9.91%) solid carcinoma. Most carcinomas were classified as grade I (36.36%) and II (45.45%). As for cytogenetic analysis, it was possible to obtain metaphases in all cultures, with good band resolution, especially in blood culture. Nineteen metaphases from lymphocytes presented 78 chromosomes, corresponding to the standard dog karyotype. The average chromosome count was under 78, hence all tumours had a near-diploid karyotype. Also, in five tumours, polyploidies were observed ranging from 142 to 157 chromosomes. Most metaphases appeared to have no structural rearrangements, but cytogenetic analysis in these female dog tumours, even with a good pattern of bands, was difficult. Future studies using molecular cytogenetic techniques will improve the cytogenetic analysis perhaps enabling a better understanding of subjacent mechanisms associated to tumorigenesis and may provide new insights, contributing to the development of new and effective therapies for humans and dogs.

### References

- <sup>1</sup>de Faria Lainetti P, Brandi A, Leis Filho AF, Prado MCM, Kobayashi PE, Laufer-Amorim R, Fonseca-Alves CE (2020). *Frontiers in Veterinary Science*, 7, 1-7.
- <sup>2</sup>Szczerbal I, Switonski M (2021). *Animals*, 11(4), 1-15.

## Is MGP involved in *Acomys cahirinus* ear regeneration?

Marta Vitorino<sup>1,2,3</sup>, Natércia Conceição<sup>1,2,3</sup>, Débora Varela<sup>1,2,3</sup>, Gustavo Tiscornia<sup>1</sup>, M. Leonor Cancela<sup>1,2,3</sup>

<sup>1</sup>Centre of Marine Sciences, University of Algarve, Faro, Portugal

<sup>2</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Algarve Biomedical Center, University of Algarve, Faro, Portugal

Email: [nconcei@ualg.pt](mailto:nconcei@ualg.pt)

The ability to regenerate damaged or missing organs has long been considered a primordial objective of modern medicine. This capacity is present in almost all metazoans. Among vertebrates, it has been reported regeneration in urodele amphibians and, to a lower extent, in teleost fish, that can regenerate several structures. In mammals, on the other hand, tissue regeneration is a rare event, responding to injury by wound healing through fibrotic scarring.

Recently, the African spiny mouse (*Acomys cahirinus*) has arisen as an emerging model of mammalian epimorphic regeneration. This animal is capable of non-fibrotic regeneration of extensive dermal wounds, including dermis, epidermis, hair follicles, sebaceous glands, and adipose tissue. Experiments previously performed in our lab showed that the spiny mice can regenerate full-thickness ear holes up to 4 mm in around 2 months and that the regenerated tissue is not a fibrotic scar but a fully developed ear pinna with normal tissue structure, including cartilage, dermis, epidermis, adipose tissue, sebaceous glands, hair, and a well-developed capillary network.

Matrix Gla protein is a vitamin K-dependent protein, involved in vascular calcification, preventing the trans-differentiation of vascular smooth muscle cells into osteoblasts, and acts as an inhibitor of cartilage calcification. MGP functions as well in regulating cell differentiation and angiogenesis.

We observed that in *A. cahirinus*, the MGP protein structure is very similar to the human homologue, containing the same structural domains.

During *A. cahirinus* ear regeneration, *MGP* is down-regulated, but in the latest stages of cell differentiation, we observed an increase in *MGP* expression suggesting that it is involved in the ear regeneration. *MGP* protein localization during the late stages of regeneration showed its expression in the epidermis, hair follicles, cartilage, and the muscle cells situated near the dorsal side of the cartilage. Altogether, these data suggest a possible role of *MGP* in the latter stages of regeneration, namely during the differentiation of the newly formed tissues.

## Preliminary data about FA-SAT ncRNA function in Mitosis

Marisa Carvalho<sup>1</sup>, Daniela Ferreira<sup>1,2</sup>, Mariana Lopes<sup>1,2</sup>, Diogo Lucas<sup>1</sup>, Gabriela Veríssimo<sup>1</sup>, Filomena Adegas<sup>1,2</sup>, Sandra Louzada<sup>1,2</sup>, Raquel Chaves<sup>1,2</sup>

<sup>1</sup>CAG - Laboratory of Cytogenomics and Animal Genomics, Department of Genetics and Biotechnology, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

<sup>2</sup>BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal

Email: [marisa-carvalho16@hotmail.com](mailto:marisa-carvalho16@hotmail.com)

For several years, satellite DNA (satDNA) sequences were considered as “junk DNA”. However, from the 2000s onwards they began to arouse interest in the scientific community. Therefore, it was possible to perceive that these sequences were transcribed, giving rise to non-coding satellite RNAs (satncRNAs)<sup>1</sup>. Recently, satellite transcripts have been associated with important roles in different cellular processes (e.g., heterochromatinization), biological contexts (e.g., response to stress conditions), and diseases (e.g., cancer)<sup>2,3</sup>.

FA-SAT is the major satellite of the domestic cat, being conserved and transcribed in several Bilateria species, including human<sup>5</sup>. FA-SAT ncRNA cellular profile was traced in cat cells, revealing accumulation of transcripts in G1 phase of the cell cycle and a nuclear location. Furthermore, it was recently showed that FA-SAT ncRNA interacts with PKM2 protein, making the switch between cell proliferation and apoptosis in both cat and human cells<sup>2,4</sup>. Also, it was possible to observe the presence of FA-SAT transcripts in mitosis in cat tumor cell lines. However, in human cells, the molecular and cellular profile of FA-SAT needs to be further investigated. Hence, we analyzed the cellular profile of FA-SAT ncRNA in human normal and cancer cell lines by RNA-FISH combined with immunofluorescence for mitosis analysis. Furthermore, an anti-mitotic drug (paclitaxel) was used in sensitive and resistant breast cancer cell lines to understand the potential role of FA-SAT ncRNA in their cell fate.

Our results are promising, showing, for the first time, the presence of FA-SAT transcripts in mitosis in human cells and their accumulation in taxol-resistant cells, as a result of their treatment. We believe that in the future, with a deeper study involving several cell lines, we could find out the role of FA-SAT ncRNA in mitosis and in the resistance of anti-mitotic drugs.

### References

- <sup>1</sup>Ferreira, et al. (2019), *Springer*, vol 77,1371-1386.
- <sup>2</sup>Ferreira, et al. (2015), *Chromosome research*, vol 23(3), 479-93.
- <sup>3</sup>Lu and Gilberto (2007), *The Journal of cell biology*, vol 179(3), 411-21.
- <sup>4</sup>Fanning (1987), *Journal of Molecular Biology*, vol 197(4), 627-34.
- <sup>5</sup>Chaves, et al. (2017), *Genome biology and evolution*, vol 9(11), 3073-3087.

## Seeing is believing: transcription profiling of Human Satellite 1

Mariana Lopes<sup>1,2</sup>, Sandra Louzada<sup>1,2</sup>, Daniela Ferreira<sup>1,2</sup>, Gabriela Veríssimo<sup>1,2</sup>, Margarida Gama-Carvalho<sup>2</sup>, Raquel Chaves<sup>1,2</sup>

<sup>1</sup>Laboratory of Cytogenomics and Animal Genomics (CAG), Department of Genetics and Biotechnology (DGB), Universidade de Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>2</sup>Biosystems and Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal Portugal

Email: [lopesmariana@gmail.com](mailto:lopesmariana@gmail.com) ; [mhcarvalho@fc.ul.pt](mailto:mhcarvalho@fc.ul.pt)

Satellite DNAs are complex, tandemly repeated, and highly organized sequences present at human centromeres and pericentromeres. In contrast to other deeply studied human satellite DNA families, human satellite 1 (HSAT1) has been dismissed from genomic and transcriptional analysis, despite being recently identified as an acrocentric key player spanning for megabase-sized stretches<sup>1</sup>. Our main goal was to assess if HSAT1 is transcribed into ncRNAs and, if so, in which conditions. With this reasoning, we decided to start by analyzing HSAT1 transcription in a wide variety of human non-tumoral and tumoral cell lines (MCF10A, GM12878, HDFn, GM03417, GM11535, GM08854, HeLa, A549, H1299, H1975, H2228, PC9, MDA-MB-231, MDA-MB-468, SK-BR-3, MCF7, Caco-2, and HepG2) and three different tissue samples (mammary, brain and small intestine). In certain contrast with reports of aberrant satellite ncRNA expression in cancer<sup>2</sup> we were able to detect different transcription profiles in cancer cell lines. By using MCF10A as a non-tumoral reference, we were able to see that some cell lines (MDA-MB-231, MDA-MB-468, H1299, and A549) have aberrant overexpression of HSAT1, others (SK-BR-3, PC9, and H2228) overexpress HSAT1 in a much smaller extent, and H1975 underexpresses HSAT1. Subsequently, single-cell analysis by RNA-FISH was performed to visualize HSAT1 ncRNAs and explore distinctive transcript features between highly expressing tumoral cell lines and non-tumoral cell lines. In a more thorough approach, HSAT1 transcripts detection was coupled with immunofluorescence and analyzed in different stages of the cell cycle in MDA-MB-231 cell line, showing an intriguing topographical distribution in mitosis.

Acknowledgements: This work was supported by the project EXPL/BIA-OUT/1028/2021 and the Scientific Employment Stimulus (CEECIND/01825/2017) from FCT (Portugal). M.L. is recipient of a fellowship (Ref SFRH/BD/147488/2019) from FCT (Portugal).

### References

<sup>1</sup>Altemose, N., Logsdon, G. A., Bzikadze, A. V., Sidhwani, P., Langley, S. A., Caldas, G. V., ... & Miga, K. H. (2022). *Science*, 376, eabl4178.

<sup>2</sup>Gao, N., Li, Y., Li, J., Gao, Z., Yang, Z., Li, Y., ... & Fan, T. (2020). *Frontiers in Oncology*, 10, 2903.

## The importance of molecular identification of parasites - Example of *Dictyocaulus* spp.

Adelina Gama<sup>1,2</sup>, Miguel Castro<sup>3</sup>, Isabel Pires<sup>1,2</sup>, Fernanda Seixas<sup>1,2</sup>, Maria de Lurdes Pinto<sup>1,2</sup>, Maria dos Anjos Pires<sup>1,2</sup>, Teresa Coutinho<sup>1</sup>, Ana Patrícia Lopes<sup>1,2</sup>, Estela Bastos<sup>3,4</sup>

<sup>1</sup>Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real

<sup>2</sup>Veterinary and Animal Science Research Center (CECAV), UTAD, Vila Real

<sup>3</sup>Department of Genetics and Biotechnology, UTAD, Vila Real

<sup>4</sup>Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB)-UTAD, Vila Real

Email: [ebastos@utad.pt](mailto:ebastos@utad.pt)

Lungworms of the genus *Dictyocaulus* (Nematoda: Trichostrongyloidea) are the causative agents of parasitic bronchitis (dictyocaulosis) of various ungulate hosts, including domestic and wild ruminants. *Dictyocaulus* adults occupy the bronchi, frequently causing subclinical and clinical disease, having impact in animal health and production <sup>[1]</sup>. The development of molecular biology techniques applied to parasitology, encompassing small subunit (SSU) and ITS2 ribosomal (r)DNA genetic and mitochondrial (mt) DNA markers has empowered advances in the genetic and epidemiological studies and in systematic of *Dictyocaulus* lungworms<sup>[2]</sup>. The genus *Dictyocaulus* is represented by several species, including *D. capreolus*.

Thirty four roe deer (*Capreolus capreolus*) were necropsied at UTAD (between 2017 and 2021). Adult lungworms were collected followed by morphological characterization. The morphological analysis showed nodular lesions and/or areas of lung consolidation in nine animals (26.5%), with one animal presenting *Dictyocaulus* spp. in the bronchi. A parasitic pneumonia was confirmed histologically in eight animals (23.5%), characterized by the presence of nematode larvae and eggs, concomitant with inflammation and smooth muscle hypertrophy. At the same time, DNA extraction was performed from two intact specimens followed by PCR amplification of a region of ITS2 that can distinguish *Dictyocaulus* species <sup>[3]</sup>. Sequencing of the amplicons was performed. The molecular method based on multiplex PCR revealed a single amplicon with a length compatible with *D. capreolus*. The sequencing of this product allowed us to analyse a sequence with 388 bp, confirmed by BLAST analysis as belonging to *D. capreolus* with the accession number MZ746962.1. It is important to validate molecular markers with potential application in systematic, population genetic or molecular epidemiological studies. The correct diagnosis of lungworm species and a deep knowledge of host ranges and transmission patterns of *Dictyocaulus* spp. are crucial for reducing the risk of cross-transmission between wildlife and livestock species.

This work was supported by the projects Project 029947IC&T 02/SAICT/2017-SAICT funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the projects UIDB/04033/2020 funded by the Portuguese Foundation for Science and Technology (FCT)

### References

- <sup>1</sup>Panuska, C., (2006). Lungworms of ruminants. Vet. Clin. North Am. Food Anim. 22 (3), 583–593.
- <sup>2</sup>Pyziel, A. M., Laskowski, Z., & Höglund, J. (2018). An assessment of the use of cox1 and cox3 mitochondrial genetic markers for the identification of *Dictyocaulus* spp. (Nematoda: Trichostrongyloidea) in wild ruminants. Parasitology research, 117(7), 2341–2345.
- <sup>3</sup>Pyziel, A. M., Laskowski, Z., & Höglund, J. (2015). Development of a multiplex PCR for identification of *Dictyocaulus* lungworms in domestic and wild ruminants. Parasitology Research, 114(10), 3923–3926.



## Pilot screen aiming to unravel novel BRCA2 gene interactions in *Drosophila melanogaster*

Soraia da Silva<sup>1</sup>, Margarida Tiago<sup>2</sup>, Torcato Martins<sup>1</sup>, Rui D. Silva<sup>2</sup>, Rui G. Martinho<sup>1,3</sup>

<sup>1</sup>Institute for Biomedicine (iBiMED), Departamento de Ciências Médicas (DCM), Universidade de Aveiro, Portugal

<sup>2</sup>Algarve Biomedical Center (ABC), Universidade do Algarve, Portugal

<sup>3</sup>Instituto de Medicina Molecular João Lobo Antunes, Universidade de Lisboa Portugal

Email: [soraias@ua.pt](mailto:soraias@ua.pt)

Breast Cancer gene 2 (BRCA2) is a DNA repair protein whose function is essential for homologous recombination repair<sup>1</sup>. Recently, it was suggested that BRCA2 also regulates transcriptional elongation<sup>2</sup>, which opened the possibility of this repair protein having additional non-canonical functions relevant for the increased cancer susceptibility of BRCA2 mutations<sup>3</sup>.

Our aim is to use *Drosophila melanogaster* as a model organism to study BRCA2 functions *in vivo* and in a developing multicellular organism. Similar to our previous work<sup>4</sup>, we performed a forward genetic screen, using the *Drosophila* larvae imaginal discs and tissue-specific RNA interference (RNAi), to identify genes whose depletion, within highly proliferative epithelial cells, genetically interact with BRCA2 RNAi.

We screened 350 *Drosophila* genes related to DNA repair/DNA replication/gene expression and chromatin remodelling machineries and identified 13 genes whose depletion within the larvae imaginal discs could produce adult wing phenotypes which could be specifically enhanced or suppressed by simultaneous depletion of BRCA2.

We will present our ongoing work related with a class of chromatin remodelling proteins whose phenotypes can be efficiently suppressed by depletion of BRCA2. Since many of these genetic interactions are likely to be conserved in humans, our work further validates the use of *Drosophila melanogaster* as a screening platform for the identification of genetic interactions relevant for cancer development and treatment.

### References

- <sup>1</sup>K. Yoshida and Y. Miki, "Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage," *Cancer Sci.*, vol. 95, no. 11, pp. 866–871, 2004, doi: 10.1111/j.1349-7006.2004.tb02195.x.
- <sup>2</sup>M. K. K. Shivji, X. Renaudin, Ç. H. Williams, and A. R. Venkitaraman, "BRCA2 Regulates Transcription Elongation by RNA Polymerase II to Prevent R-Loop Accumulation," *Cell Rep.*, vol. 22, no. 4, pp. 1031–1039, 2018, doi: 10.1016/j.celrep.2017.12.086.
- <sup>3</sup>A. R. Venkitaraman, "How do mutations affecting the breast cancer genes BRCA1 and BRCA2 cause cancer susceptibility?," *DNA Repair (Amst.)*, 2019, doi: 10.1016/j.dnarep.2019.102668.
- <sup>4</sup>R. D. Silva, M. Mirkovic, L. G. Guilgur, O. S. Rathore, and R. A. Oliveira, "Absence of the Spindle Assembly Checkpoint restores mitotic fidelity upon loss of sister chromatid cohesion," *bioRxiv*, 2018, [Online]. Available: <http://dx.doi.org/10.1101/262204>.
- <sup>5</sup>H. Lomeli and J. Castillo-Robles, "The developmental and pathogenic roles of BAF57, a special subunit of the BAF chromatin-remodeling complex," *FEBS Lett.*, vol. 590, pp. 1555–1569, 2016, doi: 10.1002/1873-3468.12201.

## Zebrafish model to study mechanisms of ectopic mineralization associated to elastinopathies

João M.A. Santos<sup>1</sup>, Rita C.R. Abreu<sup>1</sup>, Carolina Rodrigues<sup>1</sup>, Inês Borges<sup>1</sup>, Natércia Conceição<sup>1,2,3</sup>, Paulo J. Gavaia<sup>1,2</sup>, Vincent Laizé<sup>1</sup>, M. Leonor Cancela<sup>1,2,3</sup>

<sup>1</sup>Centre of Marine Sciences, University of Algarve, Faro, Portugal

<sup>2</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Algarve Biomedical Center, University of Algarve, Faro, Portugal

Email: [jmasantos@ualg.pt](mailto:jmasantos@ualg.pt)

Mutations in the human elastin gene (*ELN*) lead to elastinopathies characterized by a loss of soft tissue elasticity and the development of ectopic mineralization. The mechanisms by which loss-of-ELN function leads to ectopic mineralization remain elusive as the presence of elastin is often required at the sites of initial mineral deposition. Studying the role of ELN *in vivo* has remained challenging as *Eln* mutant mice die prematurely with multiple cardiovascular problems. The zebrafish is an emerging model with multiple validated mutant and transgenic lines used to study ectopic mineralization. In this work, we characterized two zebrafish mutants (sa17177 and sa42459) expressing truncated forms of Eln through morphometrical and histological analyses aimed at understanding whether the zebrafish *eln* mutants are appropriate to model human elastinopathies and investigate the mechanisms underlying ectopic mineralization associated with the loss of Eln function. Our data suggest that mutations in *eln* are correlated with an increased mortality and an increased incidence of bone malformations. Upon treatment with warfarin, an anticoagulant used to block the anti-mineralization action of the matrix Gla protein, *eln* mutants showed an increase in ectopic mineralization when compared to untreated control fish. In conclusion, we characterized two zebrafish mutants with a truncated form of Eln and demonstrated for the first time that loss of Eln function in zebrafish exacerbated the process of ectopic mineralization. Our work indicates that the zebrafish *eln* mutants are a good model to study elastinopathies.

This study was funded by the European Joint Programme on Rare diseases (EJP-RD) and the Foundation for Science and Technology (FCT) through projects EJPRD/0004/2020 (PhysPath-KS) and UIDB/04326/2020.



**SESSION 5**  
**BIOMEDICAL GENETICS**

## ***CDH1* locus disruption: Genome-wide functional impacts**

Carla Oliveira

i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, PT

IPATIMUP – Institute of Molecular Pathology and Immunology of the Univ. Porto, PT

FMUP – Faculty of Medicine, University of Porto, PT

P.CCC – Porto.Comprehensive Cancer Centre, Porto, PT

ERN GENTURIS – European Reference Network in Genetic Tumour Risk Syndromes - National Coordinator for Portugal

E-mail: [carlaol@ipatimup.pt](mailto:carlaol@ipatimup.pt), [carlaol@i3S.up.pt](mailto:carlaol@i3S.up.pt)

The E-cadherin cell-cell adhesion molecule, encoded by the *CDH1* gene, is a hallmark molecule in epithelial tissues. E-cadherin loss of function is classically associated with initiation and progression of epithelial tumours and a hallmark of Epithelial to mesenchymal transition (EMT). We have studied the *CDH1* locus under multiple aspects, including its impairment in the germline of patients with Hereditary Diffuse Gastric Cancer (HDGC) and associated phenotypes, its impairment by genetic and epigenetic mechanisms and the prognostic value of protein loss in sporadic gastric cancers, as well as the role of its intronic regulatory elements in healthy and cancerous cells. For this, we use both classical molecular and clinical genetics approaches, but also next generation sequencing, bioinformatics analysis, CRISPR-Cas9 and chromatin conformation analysis (4C-seq), ATAC-seq, and bioimaging in cells and tissues.

In this talk, I will present data on the most recent results of the lab focusing on a genotype-phenotype analysis in *CDH1* germline variant carriers, a clinicopathological study that identified the deleterious role of *CDH1* loss in early gastric cancer and the identification of *CDH1* intronic regulatory regions associated with control of EMT.

This work was financed through Solve-RD project [Grant agreement No 779257 - European Union's Horizon 2020 research and innovation programme]; ERN-GENTURIS; FCT/FEDER/COMPET [Grant Refs. PTDC/BTM-TEC/30164/2017; PTDC/BTM-TEC/6706/2020"]; GenomePT [Grant Ref. POCI-01-0145-FEDER-022184].

## Functional characterization of putative miRNA-like sequences encoded in the HIV genome

Carolina Ruivinho<sup>1</sup>, Andreia Jesus Amaral<sup>2,3</sup>, Ana Espada de Sousa<sup>4</sup>, Margarida Gama-Carvalho<sup>1</sup>

<sup>1</sup>BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa

<sup>2</sup>CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>3</sup>Laboratório Associado para a Ciência Animal e Veterinária (AI4Animals), Avenida da Universidade Técnica, 1300-477 Lisboa

<sup>4</sup>Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

Email: [csruivinho@fc.ul.pt](mailto:csruivinho@fc.ul.pt)

The Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the genera of lentiviruses. Recent strategies to manage HIV infection focus on miRNAs as therapeutic targets. These are small non-coding RNAs (sncRNAs) with 19-24 nucleotides long, involved in gene expression regulation. An increasing number of miRNAs encoded by the host genome are being identified with an impact on HIV infection. In contrast, viral miRNAs are not as well studied. It is generally assumed that RNA viruses can't encode miRNAs, based on the notion that presence of canonical miRNA genes would result in a useless cleavage of the viral genome and transcripts.

Previous work from the lab<sup>1</sup> used high-throughput sequencing to profile the expression of sncRNAs in *in vitro* stimulated human naïve CD4 T cells in response to HIV infection. Analysis of sequencing reads that map exclusively to the viral genome revealed the existence of putative miRNA-like molecules encoded by HIV-1 and HIV-2 (Amaral *et al*, in preparation). In this work, we aim to experimentally validate these sequences as bona-fide miRNA genes by determining if they comply with the fundamental characteristics of canonical miRNA biogenesis: Dicer-dependent processing of a precursor hairpin and association with Argonaute proteins to negatively regulate targets.

Candidate pre-miRNA hairpin sequences were cloned in an expression vector and assayed in transfected HEK cells. With the intention of testing the dependence on secondary structure formation, mutants that prevent hairpin formation were generated and assayed. Preliminary results using the stem-Loop RT-qPCR method to detect mature miRNAs reveal a difference between mutant and wild-type plasmids, suggesting that the formation of these transcripts depends on a hairpin-like secondary structure. We are currently confirming these results through hybridization-based methods, as well as testing for Dicer dependency to obtain conclusive evidence for the existence of miRNA-like molecules encoded in the HIV genome.

### References

<sup>1</sup>Amaral AJ, Andrade J, Foxall RB, et al. (2017). The EMBO Journal, v.36, 346-360.

## Epigenetic modulation of oncogenic transformation during epithelial differentiation

Laura Castro<sup>1</sup>, Yuu Kimata<sup>2</sup>, Sarah Bray<sup>3</sup>, Rui Martinho<sup>1</sup>, Torcato Martins<sup>1</sup>

<sup>1</sup>BiMED-Institute for Biomedicine, Universidade de Aveiro, Portugal

<sup>2</sup>School of Life Science and Technology (SLST), ShanghaiTech University, China

<sup>3</sup>Physiology Development and Neuroscience Department, University of Cambridge, UK

Email: [torcato@ua.pt](mailto:torcato@ua.pt)

The epithelial cell layers, through reiterative stem cell divisions, self-renew and generate a progeny with specialized functions, essential for the formation and homeostasis of human organs.

Genome-wide remodelling of the **epigenetic landscape** accompanies the **epithelial specialization process** and dictates the nuclear interpretation of the external clues, i.e., cell cycle re-entry or cell differentiation decisions depend on the predisposition of the genomic targets for being activated by the effectors of the signalling pathways. Thus, the dynamic **modulation of the epigenome** during epithelial differentiation is **highly susceptible for transformation**.

To explore how epigenetic states modulate cellular response to an oncogenic signal, we used the *Drosophila* eye imaginal disc. This single layer epithelium, during a certain period, contains three distinct cellular populations in progressive states of differentiation derived from a common origin. Using appropriate genetic tools, we performed independent manipulations of each cell population to overexpress the active form of the **evolutionary conserved oncogene** Hippo pathway transcriptional regulator **Yorkie (Yki)**<sup>1-3</sup>. Paradoxically, we found that the cells more differentiated proliferate more in response to Yki than the undifferentiated proliferative precursor cells.

To understand this paradox, we characterized the **molecular signatures** of each cell state<sup>4</sup> and found that **chromatin accessibility** varies as cells differentiate, being each cell state enriched in motifs relying on specific Transcription Factors. In particular, we found a switch of chromatin accessibility on motifs recognized by the **pioneer factor *grainy head (grh)***<sup>5</sup> and a striking correlation between enrichment on these motifs and promotion of over-proliferation by Yki. Moreover, through the analysis of genetic interactions, we found a clear synergy between Grh activity and Yki-induced proliferation even in regions previously “unresponsive” to Yki-signalling.

Our data points to a **model** where the **genome-wide epigenetic changes** by pioneer factors throughout cell differentiation generates intermediate states that **render cells liable** to oncogenic transformation.

### References

<sup>1</sup>Piccolo, S., Dupont, S. & Cordenonsi, M. The biology of YAP/TAZ: hippo signaling and beyond. *Physiol. Rev.* 94, 1287–312 (2014).

<sup>2</sup>Oh, H. & Irvine, K. D. Cooperative regulation of growth by Yorkie and Mad through bantam. *Dev. Cell* 20, 109–22 (2011).

<sup>3</sup>Oh, H. & Irvine, K. D. Cooperative Regulation of Growth by Yorkie and Mad through bantam. *Dev. Cell* 20, 109–122 (2011).

<sup>4</sup>Southall, T. D. *et al.* Cell-Type-Specific Profiling of Gene Expression and Chromatin Binding without Cell Isolation: Assaying RNA Pol II Occupancy in Neural Stem Cells. *Dev. Cell* 26, 101–112 (2013).

<sup>5</sup>Jacobs, J. *et al.* The transcription factor Grainy head primes epithelial enhancers for spatiotemporal activation by displacing nucleosomes. *Nat. Genet.* 50, 1011–1020 (2018).

## **The 2020s Tooth Fairy: from loose tooth to neuronal cell cultures, an innovative method for in vitro genetic disease modeling of a rare neurological disorder**

Carvalho S<sup>1,2</sup>, Santos JI<sup>1,3,4</sup>, Ribeiro D<sup>1,3</sup>, Moreira L<sup>1,3</sup>, Duarte AJ<sup>1,3</sup>, Encarnação M<sup>1,3</sup>, Gaspar P<sup>5</sup>, Gonçalves M<sup>1</sup>, Matos L<sup>1,3</sup>, Prata MJ<sup>4,6</sup>, Pereira de Almeida L<sup>2,7</sup>, Coutinho MF<sup>1,3</sup>, Alves S<sup>1,3</sup>

<sup>1</sup>Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal

<sup>2</sup>Faculty of Pharmacy, University of Coimbra, Portugal

<sup>3</sup>Center for the Study of Animal Science, CECA-ICETA, University of Porto, Porto, Portugal

<sup>4</sup>Biology department, Faculty of Sciences, University of Porto, Portugal

<sup>5</sup>Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal <sup>6</sup>i3S – Health research and innovation institute, University of Porto, Portugal; <sup>7</sup>CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Email: [sofia.carvalho@insa.min-saude.pt](mailto:sofia.carvalho@insa.min-saude.pt)

The development of adequate in vitro disease models is a major issue in Biomedical Genetics. Those models allow for the initial screening of novel therapeutics and help us get an insight on the cellular mechanisms that underly pathology in each case. In fact, one of the best ways to get those insights is the analysis of patient-derived cells. Yet, not every cell holds potential to recapitulate relevant disease features. For neurodegenerative diseases in particular, it is challenging to grow neuronal cultures that accurately represent them because of the obvious inability to access live neurons.

This scenario changed significantly when induced pluripotent stem cells (iPSC) were first described. From then on several differentiation protocols to generate neurons from iPSC were developed. Still, iPSC generation is a laborious/expensive protocol with significant limitations in terms of production and subsequent uses. Here we present an alternative to establish patient-derived neuronal cells in a much more expedite way. We are taking advantage of the existence of a population of multipotent stem cells (SC) in human dental pulp, the dental pulp stem cells (DPSC), to establish mixed neuronal and glial cultures for a rare neurological genetic disorder: the Sanfilippo syndrome.

Sanfilippo-derived DPSC have never been used for differentiation into specific cell types even though they represent a natural source of SC that may be used to investigate human disease especially for the infantile forms. This is a total innovation in the field and we believe it holds potential to set a new trend for investigating the cellular/gene expression changes that occur in Sanfilippo and other related diseases as it relies on a non-invasive, cost-effective approach that can be set as a routine in any lab with standard cell culture conditions. Ultimately, the same method may be applied for virtually any monogenic disorder with neurological presentation.

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

## Haplotypic Determination in Portuguese and Brazilian Patients: a founder effect in a Rare Genetic Disorder?

Marisa Encarnação<sup>1</sup>, Tatiane Hammerschmidt<sup>2</sup>, Isaura Ribeiro<sup>3</sup>, Márcia Polese-Bonato<sup>2</sup>, Lisbeth Silva<sup>1</sup>, Maria Luiza Saraiva-Pereira<sup>2</sup>, Carmen Regla Vargas<sup>2</sup>, Dulce Quelhas<sup>3</sup>, Maria João Prata<sup>4</sup>, e Sandra Alves<sup>1</sup>

<sup>1</sup>Research and Development Unit, Human Genetics Department, National Institute of Health Ricardo Jorge, Porto

<sup>2</sup>Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre – Brasil

<sup>3</sup>Unidade de Bioquímica Genética, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar e Universitário do Porto (CHUP), Porto, Portugal

Email: [marisa.encarnacao@insa.min-saude.pt](mailto:marisa.encarnacao@insa.min-saude.pt)

Niemann-Pick type C (NPC) is a neurodegenerative Lysosomal Disorder caused by loss-of-function variants in *NPC1* gene and in few cases due to pathogenic variants in *NPC2* gene<sup>1</sup>. Although the disease is monogenic, the phenotypic variation among siblings or even twins, indicates that epigenetic plays an important role in the disease complexity and clinical variability. The most part of the disease-causing variants are missense, being the most prevalent variant the p.I1061T. However, in Portugal and Brazil, the most frequent is the p.A1035V, associated with a severe infantile phenotype<sup>3-5</sup>. Since there are few studies reporting this variant, we intended to expand the research on this variant having already established a collaboration with two Institutes that diagnosed a significant part of the patients with the p.A1035V variant<sup>3,4</sup>. The aim of this collaborative work is: 1) study the haplotypes of the Portuguese and Brazilian patients carrying the variant p.A1035V. 2) study the NPC protein in patients fibroblasts homozygous for p.A1035V variant. 3) analyse undiagnosed patients with clinical and biochemical diagnosis of NPC using Next generation sequencing. We have already analysed several exonic polymorphisms and the majority present the same haplotype in the patients carrying this allele. With respect to the protein our preliminary results indicates that the protein is less degraded than the I1061T, but further studies will be necessary.

This work was partially funded by EXPL/BTM-TEC/1477/2021 (FCT), CECA and AL4Animals.

### References

- <sup>1</sup>Carstea, E.D. et al. Niemann-Pick C1 disease gene: Homology to mediators of cholesterol homeostasis. *Science* (80-. ). 1997.
- <sup>2</sup>Ribeiro, I. et al. Niemann-Pick type C disease: NPC1 mutations associated with severe and mild cellular cholesterol trafficking alterations. *Hum. Genet.* 2001.
- <sup>3</sup>Hammerschmidt, T.G. et al. Molecular and biochemical biomarkers for diagnosis and therapy monitorization of Niemann-Pick type C patients. *Int. J. Dev. Neurosci.* 2018, 66, 18–23.
- <sup>4</sup>Polese-Bonato, M. et al. Niemann-Pick Disease Type C: Mutation Spectrum and Novel Sequence Variations in the Human NPC1 Gene. *Mol. Neurobiol.* 2019, 56, 6426–6435.



## Val142Ile variant in Caucasians with cardiac transthyretin amyloidosis – the more you look the more you find

Catarina Martins da Costa<sup>1</sup>, Ana Filipa Amador<sup>1</sup>, João Calvão<sup>1</sup>, Tânia Proença<sup>1</sup>, Miguel Carvalho<sup>1</sup>, Ricardo Alves Pinto<sup>1</sup>, Catarina Amaral Marques<sup>1</sup>, André Cabrita<sup>1</sup>, Teresa Pinho<sup>1</sup>; Susana Fernandes<sup>2</sup>, Filipe Macedo<sup>1,3</sup>, Elisabete Martins<sup>1,2,3</sup>

<sup>1</sup>Centro Hospitalar Universitário de S. João, EPE, Department of Cardiology, Alameda Prof. Hernâni Monteiro, 4200-319 Porto

<sup>2</sup>Centro Hospitalar Universitário São João, EPE, Department of Pathology, Medical Genetics Service, Alameda Prof. Hernâni Monteiro, 4200-319 Porto; Portugal

<sup>3</sup>Faculty of Medicine of University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

Email: [catarinamarcosta@gmail.com](mailto:catarinamarcosta@gmail.com)

Hereditary transthyretin amyloidosis (ATTRv amyloidosis) is a cause of cardiac amyloidosis, being a heterogeneous disorder with more than 100 different transthyretin gene variants described<sup>1</sup>. ATTRv Val142Ile variant is a well-known cause of cardiac amyloidosis among African American patients and is predominantly associated with severe cardiomyopathy<sup>2</sup>. In recent years an increasing number of cases with this mutation have been reported in Caucasian patients, which raises the question if this variant is underestimated in this population. As a southwestern Europe population with a substantial African genetic background, we aimed to describe the cases of cardiac ATTRv amyloidosis due to the ATTRv Val142Ile variant identified in one centre of this region<sup>3</sup>.

We identified 3 Caucasian patients (69 years old mean age; 3 men) with the Val142Ile variant from a total of 10 cases of cardiac amyloidosis tests, performed during the last two years. Biomarkers and imaging data, including bone cardiac scintigraphy, were in accordance with ATTRv amyloidosis diagnosis. One homozygous patient died with advanced heart failure. The family screening revealed 5 new asymptomatic carriers.

Our cohort from a single Portuguese center presents a high frequency of the Val142Ile ATTRv variant (30%). Considering the growing literature, this data reinforces the importance of genetic testing in TTR cardiac amyloidosis, with a speculated higher prevalence in southwestern Europe populations<sup>4</sup>. Larger studies are needed to evaluate the real prevalence of Val142Ile ATTRv variant among Caucasians.

### References

<sup>1</sup>Coelho T, Maurer MS, Suhr OB (2013). *Curr Med Res Opin.* 29(1):63-76.

<sup>2</sup>Singh A, Geller HI, Falk RH (2017). *J Am Coll Cardiol.* 2017;69(6):757-8.

<sup>3</sup>Olalde I, Mallick S, Patterson N, Rohland N, Villalba-Mouco V, Silva M, et al (2019). *Science.* 63(6432):1230-4.

<sup>4</sup>Cappelli F, Frusconi S, Bergesio F, Grifoni E, Fabbri A, Giuliani C, et al (2016). *J Cardiovasc Med.* 17(2):122-5.

## A genomic approach to explore the mechanisms of cognitive aging

Sonya Neto<sup>1</sup>, Andreia Reis<sup>1</sup>, Miguel Pinheiro<sup>1</sup>, Nadine Santos<sup>2</sup>, Ana João Rodrigues<sup>2</sup>, Vasco Neves<sup>1</sup>, Nuno Sousa<sup>2</sup>, Manuel Santos<sup>3</sup> and Gabriela Moura<sup>1</sup>

<sup>1</sup>iBiMED&Health Sciences, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup>ICVS - School of Medicine, Campus Gualtar, University of Minho, 4710-057 Braga, Portugal

<sup>3</sup>Multidisciplinary Institute of Aging, MIA-Portugal, Faculty of Medicine, University of Coimbra, Rua Largo 2, 3º, 3000-370 Coimbra, Portugal

Aging is a highly heterogeneous process characterized by a progressive loss of physical and mental capabilities and increase risk of developing age-associated diseases. Cognitive aging is depicted by a decline in several cognitive domains, mainly executive function, memory and information processing speed<sup>1</sup>. Nevertheless, some individuals seem more susceptible to age-related cognitive deterioration which critically impacts the quality of life of people and has important socioeconomic consequences. Therefore, determining the underlining molecular basis of age-related cognitive decline is a key milestone to prevent and treat a growing aged population. Multi-omics data integration allows the study of biological process taking a more integrative approach by acknowledging the biological interrelationship within biological systems, enabling a better understanding of complex traits<sup>2</sup>. Here, we present data regarding a peripheral blood multi-omics analysis comprising genotypic, transcriptomic and methylation data of a healthy aging cohort. The cohort is composed of 443 community-dwelling individuals and is representative of the general Portuguese population regarding gender, age and education. Participants underwent a neurocognitive and psychological evaluation and MRI scans for brain gray and white matter volumetric measurements were acquired. After identifying cognitive tests that best discriminate age groups, single omics supervised multivariate analysis was used to identify: genotypic, transcriptomic, methylation, clinical and imaging variables discriminating subjects with higher and lower cognitive scores. Subsequently, canonical correlation analysis was used for multi-omic data integration. Blood transcriptomic analysis identified an enrichment of genes involved in the regulation of protein metabolism and actin cytoskeleton organization while, an enrichment of differentially methylated probes mapped to genes with transcriptional factor activity might suggest dysregulated peripheral gene expression. The identification of overlapping biological process between individual omics highlight the involvement of glucose metabolism, inflammation and synaptic dysfunction in cognitive aging. In conclusion, these findings pinpoint major mechanisms contributing to inter-individual variability of age-related cognitive alteration.

This work was supported by FCT and FEDER funds through the COMPETE 2020 (GenomePT: POCI-01-0145-FEDER-022184) and CENTRO2020 (research project with reference CENTRO-08-5864-FSE-000031). The iBiMED research unit is supported by FCT (UID/BIM/04501/2020)

### References

<sup>1</sup>Grady, C. (2012). *Nat. Rev. Neurosci.*, 13, 491–505.

<sup>2</sup>I. Subramanian, S. Verma, S. Kumar, A. Jere, K. Anamika (2020). *Bioinform. Biol. Insights.*, 31;14:1177932219899051

## A new cell proliferation control gene in *Drosophila melanogaster*

Inês Baião Santos<sup>1,2</sup>, Juan Garrido-Maraver<sup>1,3</sup>, Levente Kovács<sup>4</sup>, Álvaro Tavares<sup>1</sup>

<sup>1</sup>Departamento de Ciências Biomédicas e Biomedicina, Universidade do Algarve, 8005-139, Faro, Portugal

<sup>2</sup>Programa Doutoral em Ciências Biomédicas, Universidade do Algarve, 8005-139, Faro, Portugal

<sup>3</sup>*current affiliation*: Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Spain

<sup>4</sup>Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, California 91125

Email: [ibsantos@ualg.pt](mailto:ibsantos@ualg.pt)

Correct chromosome segregation during mitosis is critical to ensure genome integrity during cell-cycle progression. Failure of the regulatory mechanisms that detect and correct mitotic errors can lead to the accumulation of DNA damage – an hallmark of genomic instability and cancer.

In multicellular organisms, the major signalling pathway regulating the balance between cell proliferation and apoptosis is the hippo pathway. Interestingly, several components of the hippo pathway have been shown to be important regulators of mitosis, implicating new mechanisms through which the hippo pathway exerts its tumour suppressive function.

Using a CRISPR genetic approach, we generated null mutants of some of the hippo pathway regulator genes, allowing to study their function in a developmental model, *Drosophila melanogaster*.

All null mutants created turned out to be lethal except for one gene. This results in a viable but subvital phenotype, with null adult flies exhibiting tumours, melanotic masses and other morphological malformations. This phenotype is aggravated if flies are raised at 29°C (stressful rearing temperature) instead of 25°C (normal rearing temperature). Analysis of early embryos derived from these null flies shows a loss of synchronicity in the syncytial divisions associated with high levels of DNA damage. The link between the accumulation of DNA damage during the embryonic development of null mutant flies and the existence of tumours in the adult flies, is now under investigation. Importantly, expression of a GFP-tagged transgene fully rescues the null phenotype, confirming the specificity of the genetic approach employed.

The present work therefore represents the discovery of a so far unidentified tumour suppressor gene in *Drosophila melanogaster*.

This work was financed through the project PROJECTO ALG-01-0145-FEDER-030014, CRESC Algarve 2020, FCT–Fundação Ciência e Tecnologia.

## A Personalized RNA-based therapy for Mucopolidosis II: *in vitro* and *in vivo* studies

Mariana Gonçalves<sup>a,b\*</sup>, Liliana Matos<sup>a,c\*</sup>, Juliana I. Santos<sup>a,c,d</sup>, Maria Francisca Coutinho<sup>a,c</sup>, Maria João Prata<sup>d,e</sup>, Maria João Pires<sup>b</sup>, Paula Oliveira<sup>b</sup>, Sandra Alves<sup>a,c</sup>

<sup>a</sup>Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal

<sup>b</sup>Center for the research and technology of agro-environmental and biological sciences (CITAB/UTAD)

<sup>c</sup>Center for the Study of Animal Science, CECA-ICETA, University of Porto

<sup>d</sup>Biology department, Faculty of Sciences, University of Porto, Portugal

<sup>e</sup>i3S – Health research and innovation institute, University of Porto, Portugal

\*These authors contributed equally to this work.

Email: [mariana.goncalves@insa.min-saude.pt](mailto:mariana.goncalves@insa.min-saude.pt)

Mucopolidosis type II (MLII) is a Lysosomal Storage Disorder caused by the deficiency of the enzyme GlcNAc-1-phosphotransferase. This enzyme is responsible for the addition of the mannose-6-phosphate marker to lysosomal enzymes allowing their targeting to lysosomes. 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 this study, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (AOs) for MLII. In a previous study<sup>1</sup> on fibroblasts from MLII patients, AOs were used to skip exon 19 of the *GNPTAB* pre-mRNA, successfully resulting in the production of an in-frame mRNA. Currently, our objective 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* experiments, the C57BL/6 fibroblasts were transfected with AOs 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.

Then, in *in vivo* studies, 18 animals were divided into 6 groups: groups 1 and 4 were injected with saline solution, groups 2 and 5 were injected with the OA at 25 mg/kg and groups 3 and 6 were injected with the OA at 50 mg/kg. After 4- or 7-days post-treatment, the animals were sacrificed. At the end of the experiment, the organs were collected for RNA extraction, cDNA synthesis and RT-PCR. However, the exon 19 skipping was not observed. Thus, we can theorize that the doses administered were not sufficient to elicit a response or the AO may have had a high clearance rate. These are preliminary data, so in the near future more experiments will be done.

### References

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

## Approaching the mechanistic basis of disease tolerance in *Drosophila melanogaster*

Priscilla A. Akyaw<sup>1</sup>, Tânia F. Paulo<sup>1</sup>, David Duneau<sup>2</sup>, Élio Sucena<sup>1,3</sup>

<sup>1</sup>Instituto Gulbenkian de Ciência, Oeiras, Portugal

<sup>2</sup>CNRS, Laboratoire Évolution & Diversité Biologique, Université Toulouse 3 Paul Sabatier, Toulouse, France

<sup>3</sup>Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Email: [esukena@igc.gulbenkian.pt](mailto:esukena@igc.gulbenkian.pt)

Host immune defence against pathogens takes two main forms: resistance (pathogen elimination) and disease tolerance (reducing negative impact of infections without affecting pathogen load). The mechanisms controlling resistance response are well elucidated, for the most part, across different host species and consist of the activation of immune pathways that lead to pathogen killing. Disease tolerance on the other hand, pertains to mechanisms that favour the hosts ability to withstand and/or repair damage inflicted either directly by the invading pathogen (i.e., virulence or pathogen-associated injury) or damages caused indirectly by the host's own immune effectors during the process of resistance (i.e., immunopathology). In both scenarios, comparatively less is known about the mechanisms involved, their genetic bases, and how they interact during and after an infection. Thus, it is important to devise approaches allowing separate analysis of these two disease tolerance components and better understand the ecological and evolutionary implications of both and how that eventually affects the mechanisms of resistance within a population. Using the highly tractable insect model *Drosophila melanogaster*, we present a new protocol to assess its response to immunopathology as quantified through survival and reproduction after infection with the entomopathogen *Pseudomonas entomophila*. We will present data to support this approach as a means to disentangle the mechanisms of disease tolerance against immunopathology.

**Keywords** Host-pathogen interactions, *Drosophila*, Immunopathology, Disease tolerance

## Cancer-related transcription factors regulate *MGP* promoter activity

Helena Caiado<sup>1,2</sup>, Natércia Conceição<sup>1,3,4</sup> e M. Leonor Cancela<sup>1,3,4</sup>

<sup>1</sup>BIOSKEL Lab, Center of Marine Sciences (CCMAR). University of Algarve, Faro, Portugal

<sup>2</sup>ProRegeM PhD Programme in Regenerative Medicine. University of Algarve, Faro, Portugal

<sup>3</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>4</sup>Algarve Biomedical Centre, University of Algarve, Faro, Portugal

Email: [lcancela@ualg.pt](mailto:lcancela@ualg.pt) ; [nconcei@ualg.pt](mailto:nconcei@ualg.pt)

Matrix Gla Protein (MGP) is a physiological inhibitor of ectopic calcifications and a member of the vitamin K dependent protein family<sup>1</sup>. Mutations in the *MGP* gene are responsible for the Keutel syndrome, mainly characterized by abnormal calcifications in cartilage, lungs, brain, and vascular system<sup>2</sup>. In addition to its physiological role in calcification, MGP has also been implicated in tumorigenesis processes and is abnormally regulated in several types of tumors, such as cervical, ovarian, urogenital, and breast cancer, being associated with different outcomes<sup>3</sup>.

In this work we evaluated the effect of several cancer-related transcription factors known to be involved in carcinogenesis (such as Yin Yang 1 (YY1), CCAAT/ enhancer-binding proteins alpha and beta (C/EBP $\alpha$ , C/EBP $\beta$ ) and Runt-Related protein 2 (RUNX2)), in the transcriptional regulation of *MGP* promoter and their correlation with levels of *MGP* gene expression in different types of tumors available in the TCGA online database.

Results show a negative regulation of *MGP* promoter activity in the presence of YY1, C/EBP $\alpha$ , and C/EBP $\beta$ , whereas the transcription factor RUNX2 enhanced *MGP* promoter activity, suggesting a transcriptional regulation by these factors.

Analysis of the correlation between *MGP* and these transcription factors' gene expression in the tumoral tissue, showed a negative or positive effect in different types of tumors, depending on MGP being up- or downregulated in the tumoral tissue.

These results suggest that these transcription factors might have a role in *MGP* transcription regulation, thus contributing to strengthen the hypothesis of MGP having a role in cancer.

This study received national funds from the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020 (CCMAR). HC is supported by a doctoral fellowship (PD/BD/128341/2017) from FCT.

### References

<sup>1</sup>Luo, Dicy, et al 1997. Nature, 386(6).

<sup>2</sup>Munroe, Rana et al 1999. Nature Genetics, 21(1), 142–44.

<sup>3</sup>Gheorghe and Crăciun 2016. Clujul Medical, 89(3), 319–321.

## ***Cdkl5* mutant zebrafish shows skeletal and neuronal alterations mimicking human CDKL5 deficiency disorder**

Tatiana Varela<sup>1,2</sup>, Débora Varela<sup>1,2</sup>, Natércia Conceição<sup>1,3,4</sup>, M. Leonor Cancela<sup>1,3,4</sup>

<sup>1</sup>Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal

<sup>2</sup>PhD in Biomedical Sciences and Medicine, Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>4</sup>Algarve Biomedical Centre, University of Algarve, Faro, Portugal

Email: [a44770@ualg.pt](mailto:a44770@ualg.pt)

CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental condition caused by variants in cyclin-dependent kinase-like 5 (CDKL5), a kinase necessary for normal brain development/function. CDD clinical manifestations are mainly early-onset seizures and impairment of cognitive and motor skills<sup>1</sup>. Additional phenotypes comprise microcephaly, dysmorphic facial features, and scoliosis. The mechanisms underlying CDD onset are poorly understood, and existing mouse models do not fully reproduce the human pathology, making the use of alternative models, such as zebrafish, a powerful tool to study CDD<sup>2</sup>. Therefore, our goal was to characterize a *cdkl5* mutant zebrafish line (sa21938) both at the phenotype and behavioral level to validate its use as a model for CDD. *cdkl5*<sup>sa21938</sup> homozygous mutants displayed a reduced head size which is suggestive of a microcephaly phenotype, and shorter craniofacial cartilage structures suggesting an abnormal craniofacial cartilage development. Motor behavior evaluation showed that *cdkl5*<sup>sa21938</sup> embryos presented less frequency of double coiling suggesting a compromised glutamatergic neurotransmission. Locomotor behavior assessment showed that homozygous embryos swim shorter distances, indicating an impaired motor activity. Upon treatment with pentylentetrazol, a seizure behavior and an increase in the distance travelled were observed. In conclusion, homozygous *cdkl5*<sup>sa21938</sup> zebrafish reproduce several characteristics of CDD, thus validating it as a suitable animal model to better understand the physiopathology of this disorder and for the first-line screening of molecules to rescue the phenotypes observed in CDD individuals.

This work was financed through the project Million Dollar Bike Ride Grant program from the Orphan Disease Center, USA and FCT through the project UIDB/04326/2020. TV and DV are recipient of PhD fellowship from FCT

### **References**

- <sup>1</sup>Fehr et al. (2013) The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. *Eur J Hum Genet* 21,266-273.
- <sup>2</sup>Choi, et al. (2021) Zebrafish as an animal model for biomedical research. *Experimental and Molecular Medicine* 53, 310–317.

## Characterization of the expression of *ZFP36L1* gene within a context of osteoarthritis and osteoporosis pathogenesis

Mafalda Lázaro<sup>1,2</sup>, M. Leonor Cancela<sup>1,2,3</sup>, Márcio Simão<sup>1,2</sup>

<sup>1</sup>Comparative, Adaptive and Functional Skeletal Biology (BIOSKEL) lab, Centre of Marine Sciences (CCMAR), Universidade do Algarve, Faro, Portugal

<sup>2</sup>Faculty of Medicine and Biomedical Sciences, Universidade do Algarve, Faro, Portugal

<sup>3</sup>Algarve Biomedical Center (ABC), University of Algarve, Faro, Portugal

Email: [masimao@ualg.pt](mailto:masimao@ualg.pt)

It is estimated that 1.71 billion people suffer from musculoskeletal disorders worldwide. Identification of the molecular mechanisms and new targets associated with bone and cartilage tissue turnover can contribute to develop new therapeutic approaches. With this study we propose that Zinc Finger Protein 36, C3H1 Type-Like 1 (ZFP36L1), could be a main player in bone and cartilage metabolism. ZFP36L1 is an RNA binding protein (RBP) with affinity for short AU rich elements (AREs) in 3'-UTRs of mRNAs. It has been associated with mRNA destabilization and decay of short 3'-UTRs and stabilization of long 3'-UTRs rich in AU sequences leading to the formation of TIS granules in a membraneless organelle that coincide with endoplasmic reticulum surface and can regulate the availability of surface membrane proteins, like ligands and receptors. This work aimed to identify the pattern of expression of *ZFP36L1* within a context of bone loss and cartilage degradation using Gene Expression Omnibus (GEO) database. Preliminary data from our group for the analysis of *Zfp36l1* in mice with phenotype of bone loss showed a positive correlation between expression of ZFP36L1 and increase bone remodelling, suggesting a role in osteoblast and osteoclast differentiation. These results were correlated with data from studies in GEO database which showed significant downregulation of ZFP36L1 when osteoblast terminally differentiate. Within a context of low gravity, with low bone turnover, ZFP36L1 was significantly decreased and was observed a significant downregulation upon deficient osteoclast differentiation. Within cartilage metabolism, a decreased expression of ZFP36L1 was observed in hypertrophic chondrocytes terminal differentiated and while an increase in expression was observed in chondrocytes from rheumatoid arthritis patients, suggesting increase tissue remodelling as consequence of inflammatory stimulus. These results suggest that *ZFP36L1* is expressed in tissues undergoing an increase in precursor cells recruiting and metabolism turnover and its expression decreases upon terminal differentiation.

Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal, funded by FCT - Foundation for Science and Technology- through project UIDB/04326/2020



## Convergent impact of SMA and ALS disease-associated genes on protein complexes linked to neuro-muscular degeneration

Marina Garcia-Vaquero<sup>1</sup>, Marjorie Heim<sup>2</sup>, Barbara Flix<sup>3</sup>, Marcelo Pereira<sup>1</sup>, Lucile Palin<sup>2</sup>, Tânia Monteiro Marques<sup>1</sup>, Francisco R. Pinto<sup>1</sup>, Javier de Las Rivas<sup>4</sup>, Aaron Voigt<sup>3</sup>, Florence Besse<sup>2</sup> and Margarida Gama-Carvalho<sup>1</sup>

<sup>1</sup>BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, 1749-016 Lisboa, Portugal

<sup>2</sup>Université Côte d'Azur, CNRS, Inserm, Institut de Biologie Valrose, 06108 Nice, France

<sup>3</sup>Department of Neurology, Medical Faculty, RWTH Aachen University, Aachen, 52074, Germany

<sup>4</sup>Cancer Research Center (CIC-IBMCC), Universidad de Salamanca, 37007 Salamanca, Spain

Email: [mhcarvalho@fc.ul.pt](mailto:mhcarvalho@fc.ul.pt)

Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis share both phenotypic and molecular commonalities, including the fact that they can be caused by mutations in genes encoding proteins involved in RNA metabolism, namely *Smn*, *TDP-43* and *Fus*. Although this suggests the existence of common disease mechanisms, there is currently no model to explain the converging motoneuron dysfunction caused by changes in the expression of these ubiquitously expressed genes.

In this work we generated a parallel set of *Drosophila* models for adult-onset RNAi and tagged neuronal expression of the fly orthologues of the human *SMN1*, *TARDBP* and *FUS* genes (*Smn*, *TBPH* and *Caz*, respectively). We profiled nuclear and cytoplasmic bound mRNAs using a RIP-seq approach and characterized the transcriptome of the RNAi models by RNA-seq. To unravel the mechanisms underlying the common functional impact of these proteins on neuronal cells, we devised a computational approach based on the construction of a tissue-specific library of protein functional modules. Disease-relevant modules were selected by an overall impact score measuring the estimated extent of perturbation caused by each gene knockdown. Our integrative approach revealed that although each disease associated gene regulates a poorly overlapping set of transcripts, they have a concerted effect on a specific subset of protein functional modules. Most strikingly, functional annotation reveals these modules to be involved in critical cellular pathways for neurons and in particular, in neuromuscular junction function. Furthermore, selected modules are significantly enriched in orthologues of human genes linked to neuronal disease.

This work provides a new model explaining how mutations in SMA and ALS-associated disease genes linked to RNA metabolism functionally converge to cause motoneuron dysfunction. The critical functional modules identified represent interesting biomarkers and therapeutic targets given their identification in asymptomatic disease models.

## Development of a QCM-based biosensor for the detection of NSCLC biomarkers in liquid biopsies

Catarina Lino<sup>1,2</sup>, Sara Barrias<sup>1,2</sup>, Raquel Chaves<sup>1,3</sup>, Filomena Adegas<sup>1,3</sup>, Paula Martins-Lopes<sup>1,2</sup>, José Ramiro Fernandes<sup>4</sup>

<sup>1</sup>University of Trás-os-Montes e Alto Douro, School of Life Science and Environment, Department of Genetics and Biotechnology, Blocos Laboratoriais bdg, 5000-901 Vila Real, Portugal.

<sup>2</sup>BioISI - Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8 bdg, 1749-016 Lisboa, Portugal

<sup>3</sup>Laboratory of Cytogenomics and Animal Genomics (CAG), Department of Genetics and Biotechnology (DGB), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

<sup>4</sup>Chemical Centre - Vila Real (CQVR), Physics Department, School of Science and Technology, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal.

Email: [catarina.lino@outlook.com](mailto:catarina.lino@outlook.com)

Lung cancer is the main malignant cancer reported worldwide. Due to the lack of an early diagnosis, this disease also has a low survival rate<sup>1</sup>. Deletions in the Epidermal Growth Factor Receptor gene (*EGFR*) are often associated with non-small cell lung cancer (NSCLC), a common subtype of lung cancer<sup>2</sup>. The detection of such mutations provides key information for the prognosis and treatment of the disease; therefore, the identification of biomarkers is of vital importance<sup>3</sup>. The need for fast, reliable, and early detection means in NSCLC has led to the development of highly sensitive devices able to detect cancer-associated mutations. Among them, biosensors are promising alternatives to conventional detection methods and can potentially alter the way cancer is diagnosed and treated. In this study we report the development of a DNA-based biosensor, namely a quartz crystal microbalance (QCM), applied to the detection of NSCLC biomarkers, from liquid biopsy samples. The detection, as it is the case of most DNA biosensors, is based on the hybridization between a NSCLC specific probe and the sample target DNA (positive if the target harbours that specific mutation associated to NSCLC). The surface functionalization was performed with a blocking agent (dithiothreitol) and thiolated-ssDNA strands. The biosensor was able to detect specific DNA sequences in both synthetic and real samples. Aspects such as probe regeneration and QCM reutilization were also studied.

This work was financed through PulmaGENE project, reference No. NORTE-01-0247-FEDER033533r the and BioISI project (reference No. UID/MULTI/04046/2021).

### References

- <sup>1</sup>Vachani, A., Sequist, L. V., & Spira, A. (2017). *American Journal of Respiratory and Critical Care Medicine*, 195, 1150–1160.
- <sup>2</sup>Mitsudomi, T., & Yatabe, Y. (2010). *FEBS Journal*, 277, 301–308
- <sup>3</sup>John, T., Liu, G., & Tsao, M. S. (2009). *Oncogene*, 28, 14–23.

## Effect of *Cynara cardunculus* L. var. *alitis* leaves extracts in oncogenic target proteins associated to triple negative breast cancer

Helena Caiado<sup>1</sup>, Maria M. Castro<sup>1+</sup>, Teresa Brás<sup>1,2</sup>, Maria F. Duarte<sup>1,2</sup>

<sup>1</sup>Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

<sup>2</sup>MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal

\*Current address: Institute for Research and Innovation in Health, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal

Email: [helena.caiado@cebal.pt](mailto:helena.caiado@cebal.pt); [fatima.duarte@cebal.pt](mailto:fatima.duarte@cebal.pt)

Breast cancer is one of most prevalent types of cancers in Portugal, with an estimated incidence rate accounting for 11.6 percent both genders in 2020 according to the Global Cancer Observatory data<sup>1</sup>, triple-negative breast cancer (TNBC) represents around 15 percent from all the breast cancer sub-types and is mainly characterized by the absence of hormonal receptors (HER2, estrogen and progesterone), displaying high rates of cancer relapse and poor survival outcome.

Due to the nonexistence of these receptors, therapeutic approaches for this sub-type of cancer are limited to surgery, chemotherapy or radiotherapy, where unfortunately, not all the patients have a positive response or present adverse side effects allied to these treatments.

Therefore, it is of great interest the discovery of alternative therapeutic strategies, such as, the use of bioactive compounds that could ameliorate or contribute to the patient welfare.

Mediterranean specie *Cynara cardunculus* L. comprise three varieties, were *Cynara cardunculus* L. *alitis* (DC), besides to be known for the Iberian traditional cheese production<sup>2</sup> is also known by its immuno- and hepato-protective properties<sup>3</sup>.

In previous studies, it was shown the anti-proliferative effect from the lipophilic extracts derived from the *Cynara cardunculus* L. *alitis* (DC) leaves by affecting the Akt signaling pathway and cell cycle arrest, along with cell proliferation inhibition<sup>4</sup>.

In the light of these findings, the aim of this study was to evaluate the effect of ethanolic *Cynara cardunculus* L. *alitis* (DC) leaves extracts in several oncogenic targets associated to breast cancer using The Proteome Profiler Human XL Oncology Array Kit, in the triple negative MDA-MB-231 breast cancer cell line.

Results demonstrated a regulation by these ethanolic extracts in several proteins in different signaling pathways, contributing to the great potential of using bioactive compounds extracted from natural plants as a natural complement to conventional cancer therapies.

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors also acknowledge FCT for Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and Project LA/P/0121/2020 to CHANGE - Global Change and Sustainability Institute.

### References

<sup>1</sup>International Agency for Research on Cancer. Available online: [http://globocan.iarc.fr/Pages/fact\\_sheets\\_cancer.aspx](http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx) (accessed 22 May 2022).

<sup>2</sup>Veríssimo, P.; Esteves, C.; Faro, C.; Pires, E 1995. *Biotechnol. Lett.* 17. 621–626.

<sup>3</sup>Adzet, T.; Camarasa, J.; Laguna, J.C 1987. *J. Nat. Prod.* 50. 612–617.

<sup>4</sup>Ramos, P. A. B. et al.2017. *International Journal of Molecular Sciences.* 18(1).

## Exomes of consanguineous populations are a rich source to identify new pathogenic variants: the case-study of Arabian Peninsula populations

Veronica Fernandes<sup>1,2</sup>, Joana C Ferreira<sup>1,2,3</sup>, Farida Alshamali<sup>4</sup>, Luisa Pereira<sup>1,2</sup>

<sup>1</sup>IS - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal,

<sup>2</sup>IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto, Portugal

<sup>3</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

<sup>4</sup>General Department of Forensic Sciences and Criminology, Dubai Police General Headquarters, Dubai, United Arab Emirates

Email: [yfernandes@ipatimup.pt](mailto:yfernandes@ipatimup.pt)

Genomic studies have played a crucial role in enhancing knowledge on the genetic diversity across human populations and on the understanding of the genetic basis of diseases. The directed sequencing of all exons (known as whole exome sequencing, WES), and sometimes of close-by untranslated regions (UTRs), is being increasingly used in personalized clinical genetics. Arabian Peninsula (AP) was in the path of the successful out-of-Africa migration at around 60 thousand years ago, and has since been playing a main role in the crossroad between Africa, Asia and Europe. Additionally, these populations have unique properties of an extremely high consanguinity, rendering them ideally to identify new variants of pathogenic significance through the application of homozygosity mapping.

In this work, we performed WES capture on 90 AP samples, with the particularity of enriching it for the UTRs, which potentially bear important regulatory variants. This characterization contributed nearly 20,000 new variants from over 145,000 total variants. Almost half of these new variants were in UTR3, reflecting the low effort the scientific community has dedicated to cataloguing these regions. By applying several pathogenic predicting tools, we have demonstrated the high burden in potentially deleterious variants contained in AP WES. These potentially deleterious variants are especially nonsynonymous and UTR variants, many of them located in genes that have been associated mainly with neurologic disease and congenital malformations. Confirming theoretical expectations, the burden on potentially deleterious variants was as high as the consanguinity level (inferred as sum of runs of homozygosity, SROH) increased.

Our results reinforce the need to continue cataloguing the diversity in populations with high consanguinity levels, as the potentially pathogenic variants in these populations are not eliminated by genetic drift as much as in less consanguineous populations.

## Exploring U1 snRNA splicing modulation as an alternative therapy for MPS IIIC: *in vitro* and *in vivo* studies

Liliana Matos<sup>1,2\*</sup>, Mariana Gonçalves<sup>1,3\*</sup>, Juliana I. Santos<sup>1,4\*</sup>, Maria Francisca Coutinho<sup>1,2</sup>, Maria João Prata<sup>4,5</sup>, Maria João Pires<sup>3</sup>, Paula Oliveira<sup>3</sup>, Sandra Alves<sup>1,2</sup>

<sup>1</sup>Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal

<sup>2</sup>Center for the Study of Animal Science, CECA-ICETA, University of Porto

<sup>3</sup>Center for the research and technology of agro-environmental and biological sciences (CITAB/UTAD) <sup>4</sup>Biology department, Faculty of Sciences, University of Porto, Portugal

<sup>5</sup>i3S – Health research and innovation institute, University of Porto, Portugal

\*These authors contributed equally to this work.

Email: [liliana.matos@insa.min-saude.pt](mailto:liliana.matos@insa.min-saude.pt)

A significant number of mutations that change the splicing process and lead to aberrant mRNA production have been identified in Lysosomal Storage Disorders (LSDs). Mucopolysaccharidosis type IIIC (MPS IIIC) is a very rare LSD caused by mutations in the *HGSNAT* gene which encodes an enzyme involved in heparan sulphate degradation. Splicing mutations are one of the most frequent (~20%) genetic defects in MPS IIIC. Around 55% correspond to 5' splice-site (ss) mutations thus constituting a good target for splicing therapeutics. Previously, we have demonstrated in patients' fibroblasts that a modified U1snRNA vector designed to improve the definition of the *HGSNAT* exon 2 5'ss can restore splicing impaired by the mutation c.234+1G>A<sup>1</sup>.

Currently, our goal is to evaluate *in vivo* the therapeutic potential of that modified U1 snRNA by testing it in mice expressing the human splicing defect.

For this purpose, two full-length constructs were generated by cloning the wild-type (WT) or the mutated *HGSNAT* splicing-competent cassettes in the pcDNA 3.1 vector. Then, both midigenes were transfected in Hep3B and COS-7 cells reproducing the healthy control and patient cDNA's splicing pattern. Therefore, they were used to generate mice expressing the WT (c.241+1G) or mutant (c.234+1A) alleles in the liver. These mice can be used for testing the modified U1 snRNA efficacy *in vivo*. Thus, WT or mutant midigenes were administrated in mice by hydrodynamic injection following a protocol described by Balestra *et al*<sup>2</sup>. After 48h, animals were sacrificed, the liver was collected, and the molecular analysis was performed.

Preliminary results showed the expression of the mutant construct in the liver of several animals. In a near future, further tests will be carried out to optimize experimental conditions, by testing other forms of midigenes administration.

### References

<sup>1</sup>Matos L *et al.* (2014) Orphanet J Rare Dis, 9:180.

<sup>2</sup>Balestra D *et al.* (2014) J Thromb Haemost, 12(2):177–185.

## Functional characterization of putative miRNA-like sequences encoded in the HIV genome

Carolina Ruivinho<sup>1</sup>, Andreia Jesus Amaral<sup>2,3</sup>, Ana Espada de Sousa<sup>4</sup>, Margarida Gama-Carvalho<sup>1</sup>,

<sup>1</sup>BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa

<sup>2</sup>- CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

<sup>3</sup>- Laboratório Associado para a Ciência Animal e Veterinária (AI4Animals), Avenida da Universidade Técnica, 1300-477 Lisboa, <sup>3</sup>Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

E-mail: [csruivinho@fc.ul.pt](mailto:csruivinho@fc.ul.pt)

The Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the genera of lentiviruses. Recent strategies to manage HIV infection focus on miRNAs as therapeutic targets. These are small non-coding RNAs (sncRNAs) with 19-24 nucleotides long, involved in gene expression regulation. An increasing number of miRNAs encoded by the host genome are being identified with an impact on HIV infection. In contrast, viral miRNAs are not as well studied. It is generally assumed that RNA viruses can't encode miRNAs, based on the notion that presence of canonical miRNA genes would result in a useless cleavage of the viral genome and transcripts.

Previous work from the lab<sup>1</sup> used high-throughput sequencing to profile the expression of sncRNAs in *in vitro* stimulated human naïve CD4 T cells in response to HIV infection. Analysis of sequencing reads that map exclusively to the viral genome revealed the existence of putative miRNA-like molecules encoded by HIV-1 and HIV-2 (Amaral *et al*, in preparation). In this work, we aim to experimentally validate these sequences as bona-fide miRNA genes by determining if they comply with the fundamental characteristics of canonical miRNA biogenesis: Dicer-dependent processing of a precursor hairpin and association with Argonaute proteins to negatively regulate targets.

Candidate pre-miRNA hairpin sequences were cloned in an expression vector and assayed in transfected HEK cells. With the intention of testing the dependence on secondary structure formation, mutants that prevent hairpin formation were generated and assayed. Preliminary results using the stem-Loop RT-qPCR method to detect mature miRNAs reveal a difference between mutant and wild-type plasmids, suggesting that the formation of these transcripts depends on a hairpin-like secondary structure. We are currently confirming these results through hybridization-based methods, as well as testing for Dicer dependency to obtain conclusive evidence for the existence of miRNA-like molecules encoded in the HIV genome.

### References

<sup>1</sup>Amaral AJ, Andrade J, Foxall RB, et al. (2017). The EMBO Journal, v.36, 346-360.

## Genetic analysis of Optineurin variants in Paget Disease of Bone patients from the south and center of Portugal

Ana S. Alfaia<sup>1</sup>, Natércia Conceição<sup>1,2,3</sup>, Graça Sequeira<sup>3,4</sup>, Pedro Carvalho<sup>3,4</sup>, Vítor Teixeira<sup>3,4</sup>, C. Ribeiro<sup>3,4</sup>,  
M. Leonor Cancela<sup>1,2,3</sup>

<sup>1</sup>Centre of Marine Sciences, University of Algarve, Faro, Portugal

<sup>2</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Algarve Biomedical Center, University of Algarve, Faro, Portugal

<sup>4</sup>University Hospital Center of Algarve (CHUA), Rheumatology Department, Faro, Portugal

Email: [anasofiaalfaia@gmail.pt](mailto:anasofiaalfaia@gmail.pt)

Paget's bone disease (PDB) is the second most common metabolic bone disease in the world and is characterized by a deregulation of bone resorption and formation in focal areas of the skeleton. This affects the internal bone structure making bone more likely to fracture, deform and cause chronic pain. In the last years, linkage studies identified at least eight different human susceptibility loci correlated with this pathogenesis, the most prevalent being mutation P392L in sequestosome 1 (*SQSTM 1*) gene at the PDB 3 locus. However, in many cases, the patients have no

mutations in the *SQSTM 1* gene, or in other genes correlated with this disease. Our group and others linked PDB 6 locus to the development of PDB, namely the variants rs 1561570, rs 2234968, and rs 3829923 of the optineurin (*OPTN*) gene. In this study, we investigated the presence of these mutations/variants in 20 patients from the south-central region of Portugal (Alentejo and Algarve) and in 24 family relatives. In our results, we were able to replicate in percentage the prevalence of P392L mutation in our sample. It was also possible to replicate the strong statistical association of the *OPTN* rs2234968 SNP and verify a higher prevalence of the rs1561570 T allele associated with PDB in patients.

This study received national funds from the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020 (CCMAR).

## Glucose-6-Phosphate Dehydrogenase (G6PD) mutations in a cohort of Portuguese individuals

Licínio Manco<sup>a,b</sup>, Celeste Bento<sup>a,c</sup>, Luís Relvas<sup>c</sup>, Tabita Maia<sup>c</sup>, M. Letícia Ribeiro<sup>a</sup>

<sup>a</sup>Centro de Investigação em Antropologia e Saúde (CIAS), Universidade de Coimbra, Coimbra, Portugal

<sup>b</sup>Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra, Portugal

<sup>c</sup>Serviço de Hematologia Clínica, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Email: lmanco@antrop.uc.pt

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme defect in the world, affecting more than 350 million people. In Portugal, the average frequency of G6PD deficiency in males was estimated at about 0.5% and since 2000 several G6PD-deficient alleles have been identified. The main goal of this study was to describe the G6PD deficiency genotypes in a cohort of individuals of Portuguese ancestry. A total of 138 Portuguese individuals (101 males; 37 females) were enrolled in molecular diagnosis of G6PD deficiency between 1994 and 2020 at the Hematology Unity from Centro Hospitalar e Universitário de Coimbra (CHUC). Diagnosis was made based on the clinical history, hematological data and demonstration of a reduced erythrocyte G6PD activity, out of the hemolytic episode. The molecular study was done by direct Sanger sequencing or PCR-RFLP analysis. Twenty-one different *G6PD* pathogenic mutations were found. Among them, 20 were missense, causing the amino acid change, and one was an in-frame deletion in exon 10. The three most frequent mutations belong to the G6PD A (c.376A>G) African background haplotype, namely: G6PD A- (c.202G>A; p.68Val>Met) (58.6%), G6PD Betica (c.968T>C; p.323Leu>Pro) (12.1%) and G6PD Santamaria (c.542A>T; p.181Asp>Val) (4.3%). Despite the sub-Saharan background haplotype on the X-chromosome of these three most common mutations, the hemizygous subjects have non-known black ancestry. Twelve rare pathogenic mutations had been previously identified in other populations, and six rare G6PD variants were, at the best of our knowledge, only found in the Portuguese population: Mira d'Aire (c.1048G>A), Anadia (c.1193A>G), Tondela (c.11076-c.1094del), Covão do Lobo (c.1205C>A), Figueira da Foz (c.1366G>C) and Flores (c.1387C>A). Only four identified variants, Covão do Lobo (c.1205C>A), Figueira da Foz (c.1366G>C), Tondela (c.11076-c.1094del) and Sinshu (c.527A>G) were associated with chronic haemolytic anaemia (class I variants). In conclusion, this report confirms a wide molecular heterogeneity of G6PD deficiency in the Portuguese population.



## GWAS and PRS studies of human diseases: perspectives and limitations

Vasco Neves<sup>1</sup>, Sonya Neto<sup>1</sup>, Rui Marçalo<sup>1</sup>, Thaís Córdova<sup>1</sup>, Manuel A. S. Santos<sup>1,2</sup>, Gabriela M. F. R. de Moura<sup>1</sup>

<sup>1</sup>Genome Medicine Laboratory, Institute of Biomedicine - iBiMED, Department of Medical Sciences, University of Aveiro.

<sup>2</sup>Multidisciplinary Institute of Ageing - MIAPortugal, Faculty of Medicine, University of Coimbra

E-mail: vasco@ua.pt

Genome Wide Association Studies, or GWAS, are based on hundreds of thousands to millions of genetic variant simultaneous tests across the whole genome to identify genotype-phenotype associations. Since the first GWAS, more than significant ( $p < 5e-8$ ) 372000 single nucleotide (SNP)-trait pair associations have been discovered. However, these associations explain little of the expected heritability and many may only represent spurious associations. A Polygenic Risk Score can be built, being the weighted sum of the number of selected SNPs on a given trait multiplied by the effect size estimated from an independent GWAS sample. In this way it is possible to compound the small effects together and have an idea of the genetic risk for particularly susceptible patients.

Our poster highlights : 1) the important GWAS and PRS research milestones that have been achieved over the last 15 years, 2) the major limitations and 3) the potential for their application in personalized medicine. Conversely to Mendelian traits/diseases that are normally monogenic and segregate in families (rare diseases), complex diseases are polygenic and each genetic variant makes a small contribution to the trait, creating significant difficulties in their detection and characterization. Ongoing efforts attempt at reaching the lower frequency/low signal edge SNP group, which implies larger and larger cohort sizes to achieve statistical power to detect the SNPs associated with a trait. The results from these efforts remain to be seen. We will present genomic prognostic/diagnostic results, the questions being solved at present, as well as future perspectives and limitations of the GWAS and the PRSs approaches to unravel the genetic components of human complex diseases.

This work is supported FCT and COMPETE 2020 through the following projects GenomePT : POCI-01-0145-FEDER-022184, iBiMED, FCT UID/BIM/04501/2020 and PO-CENTRO2020 through project CENTRO-08-5864-FSE-000031/Capacitation for Personalized Medicine.

## Host single genetic polymorphisms (SNPs) associated with chronic vulvovaginal candidosis: a systematic review

Joana Rolo<sup>1</sup>, Carlos Gaspar<sup>1,2,3</sup>, José Martinez-de-Oliveira<sup>1</sup>, Ana Palmeira-de-Oliveira<sup>1,2,3</sup>

<sup>1</sup>CICS-UBI: Health Sciences Research Center, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal

<sup>2</sup>Faculdade de Ciências da Saúde, University of Beira Interior, Covilhã, Portugal

<sup>3</sup>Labfit-HPRD: Health Products Research and Development Lda, Covilhã, Portugal

Email: [joanarolo@fcsaude.ubi.pt](mailto:joanarolo@fcsaude.ubi.pt)

Vulvovaginal candidosis is a vaginal infection affecting millions of women worldwide. The infection, caused by a yeast of the genus *Candida*, can re-occur with some frequency, leading to chronic vulvovaginal candidosis (cVVC). There has been an effort to elucidate risk factors associated with the onset of cVVC. Several studies have also focused on the genomics and pathogenesis of *Candida* spp. in the vaginal mucosa that have contributed to further understand the physiopathology of this condition. Nonetheless, none of these efforts has produced definitive evidence to help the diagnosis of cVVC. In this work, we aim to contribute to this field by performing a systematic review to identify host single-nucleotide polymorphisms (SNPs) that could be associated with a predisposition to recurrent vulvovaginal infection by *Candida* spp.

To achieve this aim, we performed a literature search in three databases, using the string “genetic AND polymorphism AND recurrent AND vagi\* AND candida”. After duplicates’ elimination, 38 scientific papers were inspected for relevance. Of these, only 20 were related with the proposed aim and of these, only 15 corresponded to research articles or systematic reviews with meta-analysis.

We found that all studies reported SNPs in genes associated with the immune system and/or the mucosal response to fungus. Nine potential biomarkers associated with the chronicity of vulvovaginal candidosis have been identified: Dectin-1 ( $\beta$ -glucan receptor, two studies), interleukins (IL-12, IL-4, IL-22), IDO1 (Indoleamine 2,3-Dioxygenase 1), MBL (mannose-binding lectin, six studies), NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3 inflammasome), SIGLEC15 (Sialic Acid Binding Ig Like Lectin 15) and TLR2 (toll-like receptor 2).

We conclude that predisposition of the host for cVVC could be associated with SNPs in genes related with the immune response to fungal infection. However, we suggest that genome-wide association studies should be performed in order to identify novel biomarkers or strengthen these results.

## Impact of Lab-it to consolidate molecular genetics concepts in high schools within the context of Biomedical sciences

Márcio Simão<sup>1,2</sup>, Natércia Conceição<sup>1,2,3</sup>, M. Leonor Cancela<sup>1,2,3</sup>

<sup>1</sup>Comparative, Adaptive and Functional Skeletal Biology (BIOSKEL) lab, Centre of Marine Sciences (CCMAR), Universidade do Algarve, Faro, Portugal

<sup>2</sup>Faculty of Medicine and Biomedical Sciences, Universidade do Algarve, Faro, Portugal

<sup>3</sup>Algarve Biomedical Center (ABC), University of Algarve, Faro, Portugal

Email: [masimao@ualg.pt](mailto:masimao@ualg.pt)

The introduction of molecular biology concepts in high school teaching programmes all over the world has been associated with difficulties for students to understand concepts associated with the flow of genetic information in cells. The **Lab-it** project aimed to introduce hands-on laboratory activities in molecular genetics to complement the theoretical concepts taught in school. The experimental procedures applied to biomedical were contextualized in three theoretical scenarios, (i) criminal forensic investigation, (ii) prenatal genetic screenings, (iii) molecular detection and diagnostic of patients infected with bacteria carrying multi-resistance to antibiotics. The main objective of our study was to understand, students level of interest in the Lab-it practical sessions, and obtain evidence of both the positive outcomes of this initiative and which new developments could be done to improve it. Lab-it visited several high schools in the Algarve region and a total of 843 students participated in the practical sessions during the last two years. An inquiry to the students regarding the correlation of hands-on work with the discipline curriculum was very positive (classified as very good (71.9%) and good (26.6%)). Of the students that answered the inquiry, 99.3% considered that hands-on sessions contributed to a better understanding of the methods and theoretical background associated with the flow of genetic information. Regarding the impact of the session on their choice to continue into biological sciences in the university in a scale of 1 to 10, 79,6% of the students classified it to be between 7-10. About 82.7% of the students classified between 8-10 the impact of the practical session in their motivation to study for the biological classes and succeed in the exams. We concluded that students considered the participation in Lab-it sessions to be highly relevant and important to better understand the subjects taught in their biology program.

The Lab-it is part of KCITAR, a project funded by a consortium of entities: Algarve STP-Algarve Systems and Technology Partnership, Portugal 2020 program, ESF-European Social Fund and University of Algarve (GRANT\_NUMBER: ALG-08-5864-FSE-000004 K.CITAR/10IS00018).

Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal, funded by FCT - Foundation for Science and Technology-through project UIDB/04326/2020

## Integrative approach to tissue and context dependent miRNA-mRNA interactions

Tânia Monteiro Marques<sup>1</sup>, Margarida Gama-Carvalho<sup>1</sup>

<sup>1</sup>BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, 1749-016 Lisboa, Portugal

Email: [trmarques@fc.ul.pt](mailto:trmarques@fc.ul.pt)

microRNAs are small non-coding RNAs with a key role in gene expression regulation and have been implicated in several processes and diseases, from cell differentiation to cancer. These molecules act through sequence complementarity with microRNA responsive elements, which are typically located in the 3'-untranslated region of mRNAs, negatively regulating their expression. Even though the basic mechanisms for miRNA action have been described, we are still unable to efficiently predict the functional impact of these molecules. On top of this, miR function has been proposed to be regulated by endogenous competing RNAs which can compete for the binding of miRNAs and hinder their availability to bind true targets. This hypothesis adds another layer of complexity to the miRNA-mRNA relationship and suggests that the actual ability of a given miRNA to act upon a target is dynamically influenced by the transcriptome environment it is in. This implies that, when predicting targets for a miRNA, the context in which it is expressed needs to be accounted for. Here we employed an integrative bioinformatics approach to explore the large-scale genomic data available through The Cancer Genome Atlas<sup>1</sup>, which allow the study of miRNAs and mRNAs in the same context. Such data can help to understand what are the network level rules governing the interaction between miRNAs and their putative targets and also the context in which they are more likely to occur. To achieve this, paired miRNA and mRNA patient samples were obtained for both cancer and adjacent normal tissues. miRNA and mRNA were categorized according to their tissue-specificity, their expression levels, as well as the correlation between them in each tissue/condition. We are currently exploring this large network to gain insights into the tissue specific dynamics of these interactions and hopefully contribute to the improvement of the accuracy of tissue-dependent functional target prediction.

### References

<sup>1</sup>Grossman, R. L., Heath, A. P., Ferretti, V., Varmus, H. E., Lowy, D. R., Kibbe, W. A., & Staudt, L. M. (2016) *New England journal of medicine*, 375(12), 1109–1112.

## Modeling left ventricular noncompaction cardiomyopathy in cardioids

Cátia Correia<sup>1,2</sup>, Sofia M. Calado<sup>1,2</sup>, José Bragança<sup>1,2,3</sup>

<sup>1</sup>ABC-RI - Algarve Biomedical Centre Research Institute, University of Algarve

<sup>2</sup>FMCB - School of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Champalimaud Research Program, Champalimaud Centre for the Unknown, Lisbon, Portugal

Email: [a57384@ualg.pt](mailto:a57384@ualg.pt)

Shortly after gastrulation, the human heart begins its development, thus becoming the first fully formed and functional organ in the embryo<sup>1</sup>. During its early stages, the heart consists of a spongy mesh of muscle fibres and trabeculations<sup>2</sup>. This changes between weeks 12 and 18 of gestation, as the ventricular myocardium starts condensing and compacting as the endocardium surfaces solidify<sup>2</sup>. In cases where this process fails, it leaves prominent trabeculations and intertrabecular recesses<sup>2</sup>. This is characteristic of left ventricular non-compaction cardiomyopathy (LVNC), a genetic and congenital disorder with variable symptoms from asymptomatic to heart failure, arrhythmias, thromboembolic events, and sudden cardiac death<sup>2</sup>. For decades, scientists have used 2D cell cultures to study the mechanisms of cellular behaviour and disease pathogenesis and progression *in vivo*, though, for most cases, they do not represent accurately these mechanisms<sup>3</sup>. Our bodies are highly complex 3D microenvironments, consequently, 2D models fail to generate multiple cell types and there is no cell-to-cell or cell-to-extracellular matrix interactions<sup>3</sup>. These limitations can be overcome with the use of 3D cell cultures since they also possess gene expression abilities<sup>3</sup>. For instance, stem cell-derived organoids form complex structures by self-assembly that can mimic the architecture, cellular composition, and function of organ tissues<sup>1</sup>. Thus, the main goal of this project is to develop human heart organoids (hHO) from hiPSC that we have established from patients with LVNC and compare them with hHO generated from healthy controls. Currently, we have obtained beating hHO that present clear size differences between control and patients. Even though, further research is required to continue the optimisation of our protocol, our results indicate that the hHO have the potential to be good models for cardiac diseases.

### References

<sup>1</sup>Rossi G. et al (2021). *Cell Stem Cell*, 28(2), 230–240.

<sup>2</sup>Ichida F. (2020). *Journal of Cardiology*, 75, 1–9.

<sup>3</sup>Teimouri A, Yeung P, Agu R (2019). 2D vs. 3D cell culture models for in vitro topical (dermatological) medication testing. In: Mehanna RA (ed) Cell culture. *IntechOpen*.

## Modulation of HIV-1 replication and latency by miR-34c-5p

Cláudia Noronha-Estima<sup>1</sup>, Madalena Marques<sup>2</sup>, Ana Godinho-Santos<sup>2</sup>, Carolina M Conceição<sup>2</sup>,  
Ruy M Ribeiro<sup>2</sup>, Ana E Sousa<sup>2</sup>, Margarida Gama-Carvalho<sup>1</sup>

<sup>1</sup>BioISI–Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisboa, Portugal

<sup>2</sup>Instituto de Medicina Molecular João Lobo Antunes, Faculty of Medicine, University of Lisbon, Lisboa, Portugal

Email: [cfestima@fc.ul.pt](mailto:cfestima@fc.ul.pt)

The establishment of latent viral reservoirs is the biggest barrier to curing HIV. In the experimental context, the difficult distinction between HIV-infected cells with a productive versus latent infection makes it challenging to study viral reservoirs and, consequently, to identify substances or molecules that reactivate or eradicate them. Cellular miRNAs can either inhibit or promote both primary infection and latency, through the regulation of both viral and host mRNA targets. A recent study performed in our lab demonstrated the transcriptional up-regulation of miR-34c-5p in response to TCR stimulation in naive CD4<sup>+</sup> T cells<sup>1</sup>, the main targets of the HIV virus. We additionally showed that HIV infection decreases miR-34c-5p levels, while its overexpression impacts distinct viral replication processes. The cellular pathways involved remain unknown.

Different experimental models for the characterization of HIV-infected cells exhibiting a productive or latent infection have been pursued by the scientific community. One of these approaches is based on a single-round HIV-1 vector, modified to carry the coding sequencing of GFP and mCherry, under the control of the HIV-1 LTR promoter or a constitutive promoter, respectively<sup>2</sup>. Following infection and pro-viral DNA integration, cells with a productive infection will express GFP and/or mCherry, while cells with a latent infection will only exhibit red fluorescence (mCherry<sup>+</sup>).

To understand the role of miR-34c-5p in HIV-1 replication and latency in human CD4<sup>+</sup>T cells, we are taking advantage of this viral reporter coupled to overexpression or inhibition of the miR. HIV early and late reverse transcription products, 2-viral long terminal repeat circle forms (2-LTR), and integrated proviral DNA will be quantified to complement the fluorescence profile, while miR-bound mRNAs will be mapped in parallel assays. This robust approach will support the dissection of the mechanisms and pathways controlled by miR-34c-5p and identify the stages during which it influences HIV-1 replication and latency.

### References

<sup>1</sup>Amaral, A. J. *et al.* (2017) *EMBO J* 36, 346–360.

<sup>2</sup>Calvanese, V., Chavez, L., Laurent, T., Ding, S. & Verdin, E. (2013) *Virology* 446, 283–292.

## ***MTHFR* gene in health and disease – optimization of genotyping strategies**

Daniela Bernardo<sup>1</sup>, Joana Silva<sup>1</sup>, Maria dos Anjos Pires<sup>2,3</sup>, Estela Bastos<sup>1,4</sup>

<sup>1</sup>Department of Genetics and Biotechnology, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real

<sup>2</sup>Department of Veterinary Sciences, UTAD, Vila Real

<sup>3</sup>Veterinary and Animal Science Research Center (CECAV), UTAD, Vila Real

<sup>4</sup>Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB)-UTAD, Vila Real

Email: [ebastos@utad.pt](mailto:ebastos@utad.pt)

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the major circulatory form of the folate in the blood and the co-substrate for homocysteine (Hcy) remethylation to methionine<sup>1</sup>. This enzyme is codified by the *MTHFR* gene located in the human chromosome 1. Multiple variants of this gene have been reported but the most important is the rs1801133, a missense mutation that converts Alanine to Valine in the 222 aminoacid (Ala222Val), caused by a C>T transition. This variant is associated with a reduction in the activity of MTHFR enzyme leading to an increase in plasma homocysteine levels in mutant homozygous subjects (TT); in heterozygotes (CT) homocysteine levels are higher than in CC individuals<sup>2</sup>. Multiple publications highlight the association of this variant with different diseases (neurology, diabetes, psoriasis, vascular, cancers, etc)<sup>3</sup>. In the present work we present the optimization and comparison of three methodological approaches for genotyping this variant, considering three parameters: information obtained, time consumed and cost. The three methodologies are: PCR-RFLP approach with new primers, using the *Hinf I* enzyme; the sequencing of the 765 bp amplicon using our proposed primers and a realtime PCR approach using Taqman probes. The applicability of these methods will be discussed in more detail in examples of health issues as in nutrigenomics and in disease conditions, namely in cancer and in vascular and neurological disorders. The recent publications proposing that this variant may modulate the incidence and severity of COVID-19 pandemic infection will be analysed<sup>4</sup>.

### **References**

- <sup>1</sup>Friso S, Choi SW, Girelli D, et al. (2002). A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA*; 99(08):5606–5611.
- <sup>2</sup>Rozen R. (1997). Genetic predisposition to hyperhomocysteinaemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb Haemost*; 78:523e6.
- <sup>3</sup>Liew, S. C., & Gupta, E. D. (2015). Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *Eur J Med Genet*, 58(1), 1–10.
- <sup>4</sup>Ponti, G., Pastorino, L., Manfredini, M., Ozben, T., Oliva, G., Kaleci, S., Iannella, R., & Tomasi, A. (2021). COVID-19 spreading across world correlates with C677T allele of the methylenetetrahydrofolate reductase (MTHFR) gene prevalence. *J Clin Lab Anal*, 35(7), e23798.

## Oncolytic Virotherapy: The Future of Cancer Treatment

João Mota<sup>1</sup>

<sup>1</sup>Universidade de Trás-os-Montes e Alto Douro

Email: [joao\\_m\\_l\\_m@hotmail.com](mailto:joao_m_l_m@hotmail.com)

The idea that some viruses are more aggressive towards cancer cells than others is not new. Ever since the 19<sup>th</sup> century scientists have dwelled on the possibility of oncolytic virotherapy<sup>1</sup>. In the last few years, the advancements in the fields of molecular biology and virology have made oncolytic virotherapy a reality<sup>2</sup>. Oncolytic viruses are defined as genetically engineered or naturally occurring viruses that selectively replicate in and kill cancer cells without harming the normal tissues<sup>3,4</sup>. Most oncolytic viruses in use nowadays are genetically engineered by being armed with transgenes that can have different objectives<sup>3,5,6</sup>. A few viruses have been selected and are currently being used in clinical trials, the most common being the adenovirus<sup>5,6</sup>. The first and only oncolytic virotherapy to complete clinical trials and be approved for medical use was the talimogene laherparepvec (T-VEC), a herpes simplex virus type 1 (HSV-1) armed with GM-CSF, being approved by the Food and Drug Administration (FDA) in the USA<sup>1,2,3,5</sup>. Many studies have shown promising results, reducing the side effects of treatment, when compared to chemotherapies, but with improvements to be made when it comes to efficacy, particularly with cell penetration, tumour targeting and the body's immune responses<sup>1,2,6</sup>. However, with these improvements, oncolytic virotherapy can become a viable and efficient cancer treatment option with reduced discomfort to patients.

### References

- <sup>1</sup>Goradel, N. H., Baker, A. T., Arashkia, A., Ebrahimi, N., Ghorghanlu, S., & Negahdari, B. (2021). Oncolytic virotherapy: Challenges and solutions. *Current problems in cancer*, 45(1), 100639. <https://doi.org/10.1016/j.currprobcancer.2020.100639>
- <sup>2</sup>Chaurasiya, S., Fong, Y., & Warner, S. G. (2021). Oncolytic Virotherapy for Cancer: Clinical Experience. *Biomedicines*, 9(4), 419. <https://doi.org/10.3390/biomedicines9040419>
- <sup>3</sup>Fukuhara, H., Ino, Y., & Todo, T. (2016). Oncolytic virus therapy: A new era of cancer treatment at dawn. *Cancer science*, 107(10), 1373–1379. <https://doi.org/10.1111/cas.13027>
- <sup>4</sup>Cao, G. D., He, X. B., Sun, Q., Chen, S., Wan, K., Xu, X., Feng, X., Li, P. P., Chen, B., & Xiong, M. M. (2020). The Oncolytic Virus in Cancer Diagnosis and Treatment. *Frontiers in oncology*, 10, 1786. <https://doi.org/10.3389/fonc.2020.01786>
- <sup>5</sup>Mondal, M., Guo, J., He, P., & Zhou, D. (2020). Recent advances of oncolytic virus in cancer therapy. *Human vaccines & immunotherapeutics*, 16(10), 2389–2402. <https://doi.org/10.1080/21645515.2020.1723363>
- <sup>6</sup>Macedo, N., Miller, D. M., Haq, R., & Kaufman, H. L. (2020). Clinical landscape of oncolytic virus research in 2020. *Journal for immunotherapy of cancer*, 8(2), e001486. <https://doi.org/10.1136/jitc-2020-001486>



## Preliminary tests of a piezoelectric biosensor for direct sample detection

Patrícia Gatinho<sup>1,2</sup>, Raquel Chaves<sup>1,3</sup>, Filomena Adegas<sup>1,3</sup>, José R. Fernandes<sup>4</sup>, Paula Martins-Lopes<sup>1,2</sup>

<sup>1</sup>Department of Genetics and Biotechnology, School of Life Science and Environment, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

<sup>2</sup>BioISI - Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8 bldg, 1749-016 Lisboa, Portugal

<sup>3</sup>Laboratory of Cytogenomics and Animal Genomics (CAG), Department of Genetics and Biotechnology (DGB), University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

<sup>4</sup>Chemical Centre - Vila Real (CQVR), Physics Department, School of Science and Technology, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

Email: [patricia.gatinho.s@gmail.com](mailto:patricia.gatinho.s@gmail.com)

Lung cancer is amongst the most common cause of cancer deaths worldwide, mainly because of its asymptomatic status in early stages, thus usually it is only detected at an advanced stage of the disease<sup>1</sup>. NSCLC (Non-small cells lung cancer) represents about 85% of all lung cancers<sup>2</sup>.

The identification and characterization of molecular changes involved in cell transformation from normal to malignant is of critical importance, and can help to improve prevention measures, early detection, and even to monitor the efficacy of treatments. Knowledge of the patient's tumour characteristics and genetics will greatly enhance personalized prognosis and selection of the ideal treatment for each individual patient. Early diagnosis can be achieved using human fluids that can be easily assessed (e.g. blood, urine, saliva) for routine screening of specific biomarkers. In NSCLC, epidermal growth factor receptor gene (*EGFR*) mutations can be used as biomarkers<sup>3</sup>. Biosensors, which are bioanalytical devices that can provide specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor)<sup>4</sup>, can be a way to achieve a quick, cheap, and reliable result applied to patient surveillance.

Preliminary results using a quartz crystal microbalance (QCM) for direct sample detection is presented. For such purpose, the interference of synthetic plasma on the biosensor behaviour was assessed, using synthetic probes and targets. The system was also tested on a biological sample.

This work was financed through the PulmaGENE project, reference No. NORTE-01-0247-FEDER033533 and BioISI project (reference No. UID/MULTI/04046/2021).

### References

- <sup>1</sup>Nasim F, Sabath BF, Eapen GA (2019) Lung Cancer. Medical Clinics of North America 103:463–473. <https://doi.org/10.1016/j.mcna.2018.12.006>
- <sup>2</sup>Sun X, Xu S, Yang Z, et al (2020) Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors for the treatment of non-small cell lung cancer: a patent review (2014-present). Expert Opinion on Therapeutic Patents 31:223–238. <https://doi.org/10.1080/13543776.2021.1860210>
- <sup>3</sup>Ferreira D, Miranda J, Martins-Lopes P, et al (2021) Future perspectives in detecting egfr and alk gene alterations in liquid biopsies of patients with nsclc. International Journal of Molecular Sciences 22:. <https://doi.org/10.3390/ijms22083815>
- <sup>4</sup>Theâvenot DR, Toth K, Durst RA, Wilson4 GS (1999) Eletrochemical biosensors:recommended definitions and classification

## Quick SARS-CoV-2 detection based on an innovative biosensor

Alexandra Lino<sup>1</sup>, Paula Martins-Lopes<sup>1,2</sup>, Helena Gonçalves<sup>3</sup>

<sup>1</sup>BioISI - Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8 bdg, 1749-016 Lisboa, Portugal

<sup>2</sup>University of Trás-os-Montes e Alto Douro, School of Life Science and Environment, Department of Genetics and Biotechnology, Blocos Laboratoriais bdg, 5000-901 Vila Real, Portugal.

<sup>3</sup>REQUIMTE, Instituto Superior de Engenharia do Porto, Porto, Portugal

Email: [alexandra.lino@hotmail.com](mailto:alexandra.lino@hotmail.com)

The pandemic caused by SARS-CoV-2 has highlighted the importance of a fast, portable, highly sensitive, specific, and large-scale diagnostic tool for COVID-19<sup>1</sup>. Here, we report a novel biosensor that relies on the identification of SARS-CoV-2 RNA through probe hybridization. The sensing process takes less than 10 minutes, after RNA extraction; it is fast, sensitive, specific, and requires minimal lab equipment. We successfully targeted regions in the ORF1ab, E and N genes. Depending on the target sequence, the detection limit was as low as 61 pM with real samples and 0.01 µM with synthetic targets. Moreover, specificity was achieved with all probes. The performance of the biosensor was proved with synthetic targets, as well as clinical samples obtained from nasopharyngeal swabs. This biosensor is an easily adaptable device that allows the recognition of new SARS-CoV-2 variants by changing or adding a new probe. This is a major advantage due to the virus high mutation rate, which is one of the emerging challenges regarding the current pandemic<sup>2</sup>.

This work was financed through the FCT Project “Cdots Biosensing COVID19”, nº 041\_596518523, under the frame, RESEARCH COVID-19.

### References

<sup>1</sup>Azfal, A. (2017). *Journal of Advanced Research*, 26, 145–159.

<sup>2</sup>Tao, K., Tzou, P., Nouhin, J., Gupta, R., de Oliveira, T., Pond, S., Fera, D., & Shafer, W. (2021). *Nature Reviews Genetics*, 22, 757–773

## The relationship between rs 4420638 and the estimated lung age (ELA) in Portuguese elderly

Tiago Sousa<sup>1</sup>, Licínio Manco<sup>1</sup>, Rui Cruz<sup>2</sup>, Manuela Alvarez<sup>1</sup>

<sup>1</sup>Centro de Investigação em Antropologia e Saúde, Universidade de Coimbra,

<sup>2</sup>Escola Superior de Tecnologias da Saúde de Coimbra

Email: [tiago.j.r.s@gmail.com](mailto:tiago.j.r.s@gmail.com)

Estimated Lung Age (ELA) is an indicator of pulmonary function based on the volume of air exhaled in the first second (FEV1), height and sex. This physiological index decreases following the natural aging process. In addition, lifestyle, environmental exposure, and genetic inheritance can delay the advancement of lung age and contribute to a healthier aging of lung tissue. Polymorphism rs4420638 (A/G), included in the cluster APOC1/APOE, has been associated with several molecular mechanisms that accelerate biological aging of body tissues, but its impact on lung function is still unknown. In the present study, we sought to evaluate the impact of genotypes AA, GG and GA on ELA of the elderly. The sample included 270 people aged between 65 and 80 years of both sexes, living in the Region of Coimbra, located in the center of mainland Portugal. The subjects were evaluated for genotypes at the locus rs4420638, and several physiological performance indicators. The relationship with genotypes distributed by two groups, AA, and GG + GA, was inferred using a correlation analysis and a Mann-Whitney U test, performed by the IBM SPSS 25 software. Lung age was the only indicator of physiological performance that shows significant association with rs4420638 genotypes. The presence of the G allele showed a strong association with the decrease in ELA ( $p < 0.05$ ), contributing to the acceleration of pulmonary age in relation to chronological age.

## The study of a family with a pericentric inversion and the analysis in silico of the breakpoints

Patrícia Morais<sup>1,2</sup>, Filomena Adegas<sup>3,4</sup>, Marta Souto<sup>1</sup>, Regina Arantes<sup>1</sup>, Márcia Martins<sup>5,6</sup>, Osvaldo Moutinho<sup>6</sup>,  
Rosário Pinto Leite<sup>1,6</sup>

<sup>1</sup>Genetics Laboratory, Trás-os-Montes e Alto Douro Hospital Center, Vila Real, Portugal

<sup>2</sup>Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>3</sup>Laboratory of Cytogenomics and Animal Genomics, DGB - Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>3</sup>BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

<sup>5</sup>Genetics Consultation, Centro Hospitalar de Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>6</sup>Department of Women and Children, Trás-os-Montes e Alto Douro Hospital Center, Vila Real, Portugal

Email: [patriciafonsom@gmail.com](mailto:patriciafonsom@gmail.com)

The most common inversion in human occurs in enriched heterochromatic regions of chromosomes 1, 9 and 16, and are usually considered a normal variant (polymorphism) without phenotypical consequences<sup>1</sup>. However, depending on the exact location of the double strand breaks (DSB), an inversion can be pathogenic, namely if it disrupts a coding sequence. Despite the possible phenotypical implications of a carrier, an inversion may have impacts on meiotic recombination, and can lead to infertility, miscarriages, and chromosomal unbalanced offspring<sup>1,2</sup>.

We report a family study of a rare pericentric inversion involving a large chromosome 7 segment, 7p13 to q22.1 regions. This rearrangement was first detected in a prenatal diagnosis by a positive screen for trisomy 21. The study included two phenotypically normal sisters and respective fetus, in prenatal diagnosis, and all had the inversion.

In an attempt to deepen the cytogenetic analysis, an *in silico* investigation was performed, using available databases. This analysis revealed that both chromosome bands 7p13 and 7q22.1 are highly rich in coding sequences (about XX and YY genes respectively), what would suggest some clinical implications for the carriers, that were not observed, as far as we know. However, this analysis revealed a marked presence of repetitive elements, namely, transposable elements at some intergenic regions in these bands. Since no clinical phenotype was detected in this family, we suggest that the DSB may have occurred at these transposable elements' sequences.

Chromosome 7 pericentric inversion is a rare event with only four cases described in the literature, all presenting different breakpoints. Every new case of a rare inversion should be reported in order to obtain a more precise genotype / phenotype correlation, important for genetic counseling and risk evaluation.

### References

- <sup>1</sup>Gardner, R. J. M., & Amor, D. J. (2018). *Gardner and Sutherland's Chromosome Abnormalities and Genetic Counseling*. Oxford University Press.
- <sup>2</sup>Gersen, S. L., Keagle, M. B., Gersen, S., & Keagle, M. (2013). *The principles of clinical cytogenetics*. Springer.

## Urine Stem Cells as a potential *in vitro* model for Mucopolidosis type II

Luciana Moreira<sup>1,2,3</sup>, Juliana Santos<sup>1,2,3</sup>, Mariana Gonçalves<sup>1</sup>, Sofia Carvalho<sup>1,4</sup>, Marisa Encarnação<sup>1,2,3</sup>, Maria Francisca Coutinho<sup>1,2,3</sup>, Liliana Matos<sup>1,2,3</sup>, Sandra Alves<sup>1,2,3</sup>

<sup>1</sup>National Institute of Health Dr. Ricardo Jorge, Human Genetics Department, Porto, Portugal

<sup>2</sup>Center for the Study of Animal Science (CECA/ICETA), Porto, Portugal

<sup>3</sup>AL4Animals, Portugal

<sup>4</sup>Faculty of Pharmacy of University of Coimbra, Coimbra, Portugal

Email: [luciana.moreira@insa.min-saude.pt](mailto:luciana.moreira@insa.min-saude.pt)

Mucopolidosis type II (MLII; MIM 252500) is a rare autosomal recessive lysosomal storage disease (LSD) of hydrolase trafficking, caused by pathogenic variants in *GNPTAB* gene. MLII is characterized as a multi-systemic disease with prenatal or neonatal onset and fatal outcome in early childhood <sup>[1]</sup>.

Currently, there is no cure or disease-modifying treatment available, but studies on new therapeutic approaches are ongoing, including in our laboratory, using antisense oligonucleotides <sup>[2]</sup>. However, from these studies emerged the urgent need to develop new models besides the patient's fibroblasts, ideally carrying the disease-causing alleles and representing different cell types, to test these therapies and help overcome the difficulty to translate them to clinical practice.

In this sense, we recently started isolating urine stem cells (USCs) aiming to establish a new patient-specific *in vitro* model. USCs are adult stem cells with characteristics of mesenchymal stromal cells (MSCs), as they are multipotent and capable of differentiating into chondrocytes, adipocytes, osteocytes, as well as into neuronal and skeletal myogenic cells. USCs can be obtained from voided urine using a simple, inexpensive and non-invasive procedure.

Currently we are isolating control USCs from urine of healthy individuals, using a protocol adapted from <sup>[3-4]</sup>. Next, we will collaborate with medical doctors who follow MLII patients, to obtain their urine and isolate patient-specific USCs, that will be characterized and differentiated into the most relevant cell types for MLII.

Using this approach, we aim to obtain a relevant MLII *in vitro* model for investigating the mechanisms of the disease and assist in the development of new therapies, contributing to the sustainability of research and health care in this area, and with the potential to be expanded also to other LSDs.

This work was financed through the "Bolsa SPDM de Apoio à Investigação Dr. Aguinaldo Cabral" (2021-2023); CECA (UIDP/00211/2020); AL4Animals (LA/P/0059/2020).

### References:

- <sup>1</sup>Velho RV, Harms FL, Danyukova T, Ludwig NF *et al.* (2019). *Hum. Mutat.* 40, 842–864.
- <sup>2</sup>Matos L, Vilela R, Rocha M, Santos JI *et al.* (2020). *Hum. Gene Ther.* 31, 775–783.
- <sup>3</sup>Lang R, Liu G, Shi Y, Bharadwaj S *et al.* (2013). *PLoS One.* 8, e53980.
- <sup>4</sup>Zhang Y, McNeill E, Tian H, Soker S *et al.* (2008). *J. Urol.* 180, 2226–2233.

## What makes a cell responsive to oncogenes?

Laura Castro<sup>1</sup>, Rui Martinho<sup>1</sup>, Torcato Martins<sup>1</sup>

<sup>1</sup>BiMED-Institute for Biomedicine, University of Aveiro, Portugal

Email: [lauracastro@ua.pt](mailto:lauracastro@ua.pt)

A puzzling feature of cancer development is the fact that distinct cell populations, within a given tissue, can respond differently to similar oncogenic signals. This suggests that the mechanisms underlying oncogenic susceptibility may vary between cell types/states. For example, in the *Drosophila melanogaster* eye imaginal disc<sup>1,2</sup>, cells in an advanced stage of differentiation are more proliferative in response to oncogenic stimuli than the “naive” progenitor cells from which they derived. Our goal is to understand what makes a cell responsive to strong oncogenic signals, using as a model the overexpression of the constitutively active Yki oncogene<sup>3</sup>.

Our preliminary data suggest that the activity of pioneer factors, i.e. transcription factors (TFs) that bind specific DNA target sequences and open the chromatin to other TFs, are essential for cellular response to oncogenic signals<sup>4,5</sup>, posing the hypothesis that the chromatin status is crucial for the correct interpretation of oncogenic clues. To explore how chromatin modulations mediate oncogenesis, we established a genetic screen to test whether depletion of different **chromatin remodelers** by RNAi can regulate Yki activation in different regions of the *Drosophila's* eye primordium.

We found strong synergies between modulation of chromatin and Yki-induced overgrowth that can be classified into two main classes according to the effect on retina development, (i)over-proliferation; (ii)differentiation defects. Histological analysis of both classes showed that the neuronal photoreceptors are properly differentiated, however the supportive cells either over-proliferate or fail to acquire their terminal fate. Interestingly, RNAis for some chromatin modulators seem have region specific effects as while they strongly enhance Yki-induced in intermediate cell differentiation states, they fail to produce any noticeable effect when induced in cells in a more advanced stage of differentiation.

Our results point to a local regulation of chromatin status by chromatin remodelers to control cellular response to oncogenic signals and terminal differentiation.

### References

- <sup>1</sup>C. Polesello, F. Roch, V. Gobert, M. Haenlin, and L. Waltzer, *Modeling cancers in Drosophila*, vol. 100. 2011.
- <sup>2</sup>E. Wittkorn, A. Sarkar, K. Garcia, M. Kango-singh, and A. Singh, “The Hippo pathway effector Yki downregulates Wg signaling to promote retinal differentiation in the *Drosophila* eye,” pp. 2002–2013, 2015, doi: 10.1242/dev.117358.
- <sup>3</sup>S. Song, H. Herranz, and S. M. Cohen, “The chromatin remodeling BAP complex limits tumor-promoting activity of the Hippo pathway effector Yki to prevent neoplastic transformation in *Drosophila* epithelia,” *DMM Dis. Model. Mech.*, vol. 10, no. 10, pp. 1201–1209, Oct. 2017, doi: 10.1242/dmm.030122.
- <sup>4</sup>E. D. Larson, A. J. Marsh, and M. M. Harrison, “Pioneering the developmental frontier,” *Mol. Cell*, vol. 81, no. 8, pp. 1640–1650, Apr. 2021, doi: 10.1016/j.molcel.2021.02.020.
- <sup>5</sup>R. E. Hillmer and B. A. Link, “The Roles of Hippo Signaling Transducers Yap and Taz in Chromatin Remodeling,” *Cells*, vol. 8, no. 5, p. 502, May 2019, doi: 10.3390/cells8050502.

## ZNF687 Gene Expression and Methylation in Osteoblastogenesis

Débora Varela<sup>1,2</sup>, Tatiana Varela<sup>1,2</sup>, Natércia Conceição<sup>1,3,4</sup>, M. Leonor Cancela<sup>1,3,4</sup>

<sup>1</sup>Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal

<sup>2</sup>PhD in Biomedical Sciences and Medicine, Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>3</sup>Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal

<sup>4</sup>Algarve Biomedical Centre, University of Algarve, Faro, Portugal

Email: [a44771@ualg.pt](mailto:a44771@ualg.pt)

*ZNF687* gene encodes a zinc finger protein that is expressed in most tissues, including bone. Variants in *ZNF687* are associated with a severe form of Paget's Disease of Bone (PDB), characterized by an increased bone resorption (osteoclasts) followed by an abnormal/excessive bone formation (osteoblasts). Although *ZNF687* function has been poorly explored, studies indicate that it might have a role in bone metabolism. *ZNF687* is highly expressed during the regeneration of zebrafish caudal fins and overexpressed in PBMCs from PDB patients<sup>1</sup>. Emerging evidence suggest that disturbances in epigenetic mechanisms can affect activity of bone cells and contribute to the pathogenesis of bone diseases<sup>2</sup>. Therefore, our aim was to investigate the expression and methylation of *Znf687* throughout osteoblast differentiation. For that, mouse-derived osteoblast precursor cell line (MC3T3-E1) was differentiated into mature osteoblasts upon treatment with an osteogenic cocktail. At specific differentiation times, mineralization was detected by alizarin red staining, total RNA and gDNA were extracted and levels of *Znf687* gene expression and methylation were determined through qPCR. Our results showed a higher expression of bone markers during the osteogenic treatment compared to the control. *Znf687* expression was downregulated throughout osteoblast differentiation, i.e., its expression was significantly lower in differentiated osteoblasts compared to their undifferentiated precursors. In both conditions, seven CpGs were hypomethylated and one CpG was hypermethylated. There were no differences in the *Znf687* methylation levels between the differentiated osteoblasts and the undifferentiated cells. Our work suggests that *ZNF687* plays a role in osteoblast differentiation and DNA methylation might not be involved in *Znf687* regulation. More studies are needed to understand the mechanisms involved in *Znf687* expression during this process.

This work received national funds from FCT through the project UIDB/04326/2020 (CCMAR). DV and TV are recipient of PhD fellowship from FCT.

### References

- <sup>1</sup>Divisato G, et al. (2016). ZNF687 Mutations in Severe Paget Disease of Bone Associated with Giant Cell Tumor. *Am J Hum Genet*, 98,275-86.
- <sup>2</sup>Park-Min K. (2017). Epigenetic Regulation of Bone cells. *Connect Tissue Res*, 58, 76–89.

## Exploring pivotal residues for the substrate specificity of the Dis3L2 ribonuclease

Susana M.Costa<sup>1</sup>, Cecília M.Arraiano<sup>1</sup>, Rute G.Matos<sup>1</sup>, Sandra C.Viegas<sup>1</sup>

<sup>1</sup>Control of Gene Expression Lab, ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Email: [smma.costa@itqb.unl.pt](mailto:smma.costa@itqb.unl.pt)

In humans, there are three members of the RNase II/RNB family of 3'-5' exoribonucleases, which can be distinguished according to the sequence conservation of their active site: Dis3, Dis3L (Dis3L1), and Dis3L2.

There is a strong link between hDIS3L2 and human disease. Dis3L2 is involved in several cellular mechanisms (e.g. apoptosis, cellular differentiation and proliferation), and mutations in the enzyme have been associated with Perlman Syndrome and Wilms Tumor formation in children. Distinct studies focusing on Dis3L2 unraveled a novel eukaryotic 3'-5' RNA decay pathway that challenged the previously established models. The first insight on the uridylation involvement in controlling the stability of poly(A)-containing mRNAs was reported in *Schizosaccharomyces pombe*, where it was shown that Dis3L2 activity is stimulated by the addition of untemplated uridine residues to the RNAs 3'-end. However, the precise mechanism of action of this enzyme is not yet fully understood.

Therefore, this work aims to characterize the amino acid residues that distinguish Dis3L2 from its family homologs regarding its substrate specificities, namely the preference for uridine residues. This approach will help us understand its mechanism of action and function in different eukaryotic cells.

In this work, the fission yeast protein (SpDis3L2) was used to construct mutant versions with single amino acid substitutions whose expression was induced in *E. coli*. The purified proteins were used in several assays to assess their exonucleolytic activity over different radioactively labeled RNA substrates. We have already purified active SpDis3L2 mutants and tested their preference for poly(U), using uridylated and non-uridylated endogenous substrates, and furthermore their ability to cut double-stranded RNAs. Different substitutions in SpDis3L2 had different effects on protein purification yield, and subsequently on its stability, activity, and preference for uridylated RNAs.





## Plenary Lecture Speakers

Name	Email
Timothy Sharbel	<a href="mailto:tim.sharbel@usask.ca">tim.sharbel@usask.ca</a>
Wolfgang Link	<a href="mailto:wlink222@gmail.com">wlink222@gmail.com</a>

## Invited Lecture Speakers

Name	Email
Carla Oliveira	<a href="mailto:carlaol@i3s.up.pt">carlaol@i3s.up.pt</a>
Catarina Ginja	<a href="mailto:catarinaginja@cibio.up.pt">catarinaginja@cibio.up.pt</a>
Franziska Turck	<a href="mailto:turck@mpipz.mpg.de">turck@mpipz.mpg.de</a>
Lounès Chikhi	<a href="mailto:chikhi@igc.gulbenkian.pt">chikhi@igc.gulbenkian.pt</a>
Manuel Mota	<a href="mailto:mmota@uevora.pt">mmota@uevora.pt</a>
Raquel Sá-Leão	<a href="mailto:rsaleao@itqb.unl.pt">rsaleao@itqb.unl.pt</a>

## List of Participants

Name	Email
Agostinho Antunes	<a href="mailto:aantunes@ciimar.up.pt">aantunes@ciimar.up.pt</a>
Alexandra Camelo	<a href="mailto:alexandra.camelo@cataa.pt">alexandra.camelo@cataa.pt</a>
Alexandra Rodrigues Lino	<a href="mailto:lino.alexandra@hotmail.com">lino.alexandra@hotmail.com</a>
Aliana Vairinhos	<a href="mailto:alianavairinhos@gmail.com">alianavairinhos@gmail.com</a>
Ana Alexandre	<a href="mailto:anaalex@uevora.pt">anaalex@uevora.pt</a>
Ana Alinho	<a href="mailto:ana.alinho93@gmail.com">ana.alinho93@gmail.com</a>
Ana Campilho	<a href="mailto:anacampilho@cibio.up.pt">anacampilho@cibio.up.pt</a>
Ana Faustino	<a href="mailto:ana.faustino@cebal.pt">ana.faustino@cebal.pt</a>
Ana Faustino	<a href="mailto:anafastino@uevora.pt">anafastino@uevora.pt</a>
Ana Magalhães	<a href="mailto:acmagalhaes@ipatimup.pt">acmagalhaes@ipatimup.pt</a>
Ana Margarida Ferro	<a href="mailto:ana.ferro@nyu.edu">ana.ferro@nyu.edu</a>
Ana Milhinhos	<a href="mailto:milhinho@itqb.unl.pt">milhinho@itqb.unl.pt</a>
Ana Morais	<a href="mailto:patriciafonsom@gmail.com">patriciafonsom@gmail.com</a>
Ana Rita Bezerra	<a href="mailto:armbezerra@ua.pt">armbezerra@ua.pt</a>
Ana Sofia Carvalho	<a href="mailto:aacarvalho@icbas.up.pt">aacarvalho@icbas.up.pt</a>
Ana Sofia de Alfaia	<a href="mailto:anasofiaalfaia@gmail.com">anasofiaalfaia@gmail.com</a>
Ana Usié Chimenos	<a href="mailto:ana.usie@cebal.pt">ana.usie@cebal.pt</a>
André Filipe Albuquerque	<a href="mailto:andrealb@uevora.pt">andrealb@uevora.pt</a>
André Mendes	<a href="mailto:mrandremendes@gmail.com">mrandremendes@gmail.com</a>
André Santos	<a href="mailto:afalipiosantos@gmail.com">afalipiosantos@gmail.com</a>
Andreia Amaral	<a href="mailto:andreiaamaral@fmv.ulisboa.pt">andreiaamaral@fmv.ulisboa.pt</a>
Antónia Monteiro	<a href="mailto:antonia.monteiro@nus.edu.sg">antonia.monteiro@nus.edu.sg</a>
António Nunes	<a href="mailto:antonio.nunes.mes@gmail.com">antonio.nunes.mes@gmail.com</a>
Beatriz Gomes	<a href="mailto:beatrizgo1306@gmail.com">beatrizgo1306@gmail.com</a>

Bernardo Raimundo [bernardolucas1999@gmail.com](mailto:bernardolucas1999@gmail.com)  
Bibiana Ferreira [bibiana.i.ferreira@gmail.com](mailto:bibiana.i.ferreira@gmail.com)  
Bruno Casimiro [casimiro19@gmail.com](mailto:casimiro19@gmail.com)  
Carla Oliveira [carlaol@ipatimup.pt](mailto:carlaol@ipatimup.pt)  
Carla Varanda [carlavaranda@uevora.pt](mailto:carlavaranda@uevora.pt)  
Carolina Ruivinho [csruivinho@fc.ul.pt](mailto:csruivinho@fc.ul.pt)  
Catarina Campos [mccampos@uevora.pt](mailto:mccampos@uevora.pt)  
Catarina da Costa [catarinamarcosta@gmail.com](mailto:catarinamarcosta@gmail.com)  
Catarina Milheiro [cicm@itqb.unl.pt](mailto:cicm@itqb.unl.pt)  
Catarina Rodrigues Lino [catarina.lino@outlook.com](mailto:catarina.lino@outlook.com)  
Cátia Dias Correia [a57384@ualg.pt](mailto:a57384@ualg.pt)  
Célia Miguel [cmmiguel@ciencias.ulisboa.pt](mailto:cmmiguel@ciencias.ulisboa.pt)  
Christophe Espírito Santo [cespiritosanto@cataa.pt](mailto:cespiritosanto@cataa.pt)  
Cláudia Marinho [cclaudia.cmarinho@gmail.com](mailto:cclaudia.cmarinho@gmail.com)  
Cláudia Noronha Estima [cfestima@fc.ul.pt](mailto:cfestima@fc.ul.pt)  
Claudia Vicente [cvicente@uevora.pt](mailto:cvicente@uevora.pt)  
Cristina Mendes [cristina.ib.mendes@gmail.com](mailto:cristina.ib.mendes@gmail.com)  
Daniel Bailote [m48156@alunos.uevora.pt](mailto:m48156@alunos.uevora.pt)  
Daniela Cordeiro [danielacordeiro@outlook.pt](mailto:danielacordeiro@outlook.pt)  
Débora Varela [a44771@ualg.pt](mailto:a44771@ualg.pt)  
Diana Lousa [dlousa@itqb.unl.pt](mailto:dlousa@itqb.unl.pt)  
Diogo Marques [diogo.msmarques@hotmail.com](mailto:diogo.msmarques@hotmail.com)  
Duarte Marques [duartemarques@ua.pt](mailto:duartemarques@ua.pt)  
Élio Sucena [esucena@igc.gulbenkian.pt](mailto:esucena@igc.gulbenkian.pt)  
Estela Bastos [ebastos@utad.pt](mailto:ebastos@utad.pt)  
Fátima Duarte [fatima.duarte@cebal.pt](mailto:fatima.duarte@cebal.pt)  
Gil Carraco [gdcarraco@ualg.pt](mailto:gdcarraco@ualg.pt)  
Guilherme Martins [gdc.martins@itqb.unl.pt](mailto:gdc.martins@itqb.unl.pt)  
Helena Caiado [helenacaiado86@gmail.com](mailto:helenacaiado86@gmail.com)  
Hélia Cardoso [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)  
Herlander Azevedo [hazevedo@cibio.up.pt](mailto:hazevedo@cibio.up.pt)  
Inês Baião Santos [ibsantos@ualg.pt](mailto:ibsantos@ualg.pt)  
Inês Gonçalves [ic.goncalves@fct.unl.pt](mailto:ic.goncalves@fct.unl.pt)  
Inês Vieira [m44705@alunos.uevora.pt](mailto:m44705@alunos.uevora.pt)  
Isabel Gordo [dzwerschke@igc.gulbenkian.pt](mailto:dzwerschke@igc.gulbenkian.pt)  
Isabel Sá-Nogueira [isn@fct.unl.pt](mailto:isn@fct.unl.pt)  
Isabela Vera dos Anjos [iveradosanjos@hotmail.com](mailto:iveradosanjos@hotmail.com)  
Ivo Chelo [immchelo@fc.ul.pt](mailto:immchelo@fc.ul.pt)  
Jacqueline Santos [s.jacquelineoliveira@gmail.com](mailto:s.jacquelineoliveira@gmail.com)  
Joana Ribeiro [joanaar@uevora.pt](mailto:joanaar@uevora.pt)  
Joana Rolo [joanarolo@fcsaude.ubi.pt](mailto:joanarolo@fcsaude.ubi.pt)  
João Faia Mendes [l40598@alunos.uevora.pt](mailto:l40598@alunos.uevora.pt)  
João Mota [joao\\_m\\_l\\_m@hotmail.com](mailto:joao_m_l_m@hotmail.com)  
João Pedro Martins dos Reis [a61179@ualg.pt](mailto:a61179@ualg.pt)  
João Santos [jmasantos@ualg.pt](mailto:jmasantos@ualg.pt)  
Jorge Pereira [jcpereira@utad.pt](mailto:jcpereira@utad.pt)  
José Bessa [jose.bessa@ibmc.up.pt](mailto:jose.bessa@ibmc.up.pt)  
Juliana Lopes [julianamainenti@gmail.com](mailto:julianamainenti@gmail.com)  
Lara Norris Cebolla [capitanole@gmail.com](mailto:capitanole@gmail.com)  
Laura Castro [lauracastro@ua.pt](mailto:lauracastro@ua.pt)  
Leandro Bastos Pires [leombpires@gmail.com](mailto:leombpires@gmail.com)  
Lénia Rodrigues [lrodrigues-88@hotmail.com](mailto:lrodrigues-88@hotmail.com)  
Leonor Cancela [lcancela@ualg.pt](mailto:lcancela@ualg.pt)

Bernardo Raimundo [bernardolucas1999@gmail.com](mailto:bernardolucas1999@gmail.com)  
Bibiana Ferreira [bibiana.i.ferreira@gmail.com](mailto:bibiana.i.ferreira@gmail.com)  
Bruno Casimiro [casimiro19@gmail.com](mailto:casimiro19@gmail.com)  
Carla Oliveira [carlaol@ipatimup.pt](mailto:carlaol@ipatimup.pt)  
Carla Varanda [carlavaranda@uevora.pt](mailto:carlavaranda@uevora.pt)  
Carolina Ruivinho [csruivinho@fc.ul.pt](mailto:csruivinho@fc.ul.pt)  
Catarina Campos [mccampos@uevora.pt](mailto:mccampos@uevora.pt)  
Catarina da Costa [catarinamarcosta@gmail.com](mailto:catarinamarcosta@gmail.com)  
Catarina Milheiro [cicm@itqb.unl.pt](mailto:cicm@itqb.unl.pt)  
Catarina Rodrigues Lino [catarina.lino@outlook.com](mailto:catarina.lino@outlook.com)  
Cátia Dias Correia [a57384@ualg.pt](mailto:a57384@ualg.pt)  
Célia Miguel [cmmiguel@ciencias.ulisboa.pt](mailto:cmmiguel@ciencias.ulisboa.pt)  
Christophe Espírito Santo [cespiritosanto@cataa.pt](mailto:cespiritosanto@cataa.pt)  
Cláudia Marinho [cclaudia.cmarinho@gmail.com](mailto:cclaudia.cmarinho@gmail.com)  
Cláudia Noronha Estima [cfestima@fc.ul.pt](mailto:cfestima@fc.ul.pt)  
Claudia Vicente [cvicente@uevora.pt](mailto:cvicente@uevora.pt)  
Cristina Mendes [cristina.ib.mendes@gmail.com](mailto:cristina.ib.mendes@gmail.com)  
Daniel Bailote [m48156@alunos.uevora.pt](mailto:m48156@alunos.uevora.pt)  
Daniela Cordeiro [danielacordeiro@outlook.pt](mailto:danielacordeiro@outlook.pt)  
Débora Varela [a44771@ualg.pt](mailto:a44771@ualg.pt)  
Diana Lousa [dlousa@itqb.unl.pt](mailto:dlousa@itqb.unl.pt)  
Diogo Marques [diogo.msmarques@hotmail.com](mailto:diogo.msmarques@hotmail.com)  
Duarte Marques [duartemarques@ua.pt](mailto:duartemarques@ua.pt)  
Élio Sucena [esucena@igc.gulbenkian.pt](mailto:esucena@igc.gulbenkian.pt)  
Estela Bastos [ebastos@utad.pt](mailto:ebastos@utad.pt)  
Fátima Duarte [fatima.duarte@cebal.pt](mailto:fatima.duarte@cebal.pt)  
Gil Carraco [gdcarraco@ualg.pt](mailto:gdcarraco@ualg.pt)  
Guilherme Martins [gdc.martins@itqb.unl.pt](mailto:gdc.martins@itqb.unl.pt)  
Helena Caiado [helenacaiado86@gmail.com](mailto:helenacaiado86@gmail.com)  
Hélia Cardoso [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)  
Herlander Azevedo [hazevedo@cibio.up.pt](mailto:hazevedo@cibio.up.pt)  
Inês Baião Santos [ibsantos@ualg.pt](mailto:ibsantos@ualg.pt)  
Inês Gonçalves [ic.goncalves@fct.unl.pt](mailto:ic.goncalves@fct.unl.pt)  
Inês Vieira [m44705@alunos.uevora.pt](mailto:m44705@alunos.uevora.pt)  
Isabel Gordo [dzwerschke@igc.gulbenkian.pt](mailto:dzwerschke@igc.gulbenkian.pt)  
Isabel Sá-Nogueira [isn@fct.unl.pt](mailto:isn@fct.unl.pt)  
Isabela Vera dos Anjos [iveradosanjos@hotmail.com](mailto:iveradosanjos@hotmail.com)  
Ivo Chelo [immchelo@fc.ul.pt](mailto:immchelo@fc.ul.pt)  
Jacqueline Santos [s.jacquelineoliveira@gmail.com](mailto:s.jacquelineoliveira@gmail.com)  
Joana Ribeiro [joanaar@uevora.pt](mailto:joanaar@uevora.pt)  
Joana Rolo [joanarolo@fcsaude.ubi.pt](mailto:joanarolo@fcsaude.ubi.pt)  
João Faia Mendes [l40598@alunos.uevora.pt](mailto:l40598@alunos.uevora.pt)  
João Mota [joao\\_m\\_l\\_m@hotmail.com](mailto:joao_m_l_m@hotmail.com)  
João Pedro Martins dos Reis [a61179@ualg.pt](mailto:a61179@ualg.pt)  
João Santos [jmasantos@ualg.pt](mailto:jmasantos@ualg.pt)  
Jorge Pereira [jcpereira@utad.pt](mailto:jcpereira@utad.pt)  
José Bessa [jose.bessa@ibmc.up.pt](mailto:jose.bessa@ibmc.up.pt)  
Juliana Lopes [julianamainenti@gmail.com](mailto:julianamainenti@gmail.com)  
Lara Norris Cebolla [capitanole@gmail.com](mailto:capitanole@gmail.com)  
Laura Castro [lauracastro@ua.pt](mailto:lauracastro@ua.pt)  
Leandro Bastos Pires [leombpires@gmail.com](mailto:leombpires@gmail.com)  
Lénia Rodrigues [lrodrigues-88@hotmail.com](mailto:lrodrigues-88@hotmail.com)  
Leonor Cancela [lcancela@ualg.pt](mailto:lcancela@ualg.pt)

Licínio Manco	<a href="mailto:Imanco@antrop.uc.pt">Imanco@antrop.uc.pt</a>
Liliana da Silva Matos	<a href="mailto:liliana.matos@insa.min-saude.pt">liliana.matos@insa.min-saude.pt</a>
Luciana Moreira	<a href="mailto:luciana.moreira@insa.min-saude.pt">luciana.moreira@insa.min-saude.pt</a>
Luísa Mesquita Pereira	<a href="mailto:luisap@ipatimup.pt">luisap@ipatimup.pt</a>
Manuel Martins Cavaco	<a href="mailto:m.cavaco98@hotmail.com">m.cavaco98@hotmail.com</a>
Márcia Carvalho	<a href="mailto:soraiais@ua.pt">soraiais@ua.pt</a>
Márcio Simão	<a href="mailto:masimao@ualg.pt">masimao@ualg.pt</a>
Margarida Espada	<a href="mailto:mespada@uevora.pt">mespada@uevora.pt</a>
Margarida Gama Carvalho	<a href="mailto:mhcarvalho@fc.ul.pt">mhcarvalho@fc.ul.pt</a>
Margarida Veloso	<a href="mailto:mveloso@medicina.ulisboa.pt">mveloso@medicina.ulisboa.pt</a>
Maria Confraria Gaspar	<a href="mailto:mariatxi.gaspar@gmail.com">mariatxi.gaspar@gmail.com</a>
Maria Doroteia Campos	<a href="mailto:mdcc@uevora.pt">mdcc@uevora.pt</a>
Maria Fernandes Félix	<a href="mailto:mrff@uevora.pt">mrff@uevora.pt</a>
Maria Francisca Coutinho	<a href="mailto:francisca.coutinho@insa.min-saude.pt">francisca.coutinho@insa.min-saude.pt</a>
Maria Inês Bernardino	<a href="mailto:francisca.coutinho@insa.min-saude.pt">francisca.coutinho@insa.min-saude.pt</a>
Maria J Carvalho	<a href="mailto:mjcarvalho@ua.pt">mjcarvalho@ua.pt</a>
Maria Manuela Alvarez	<a href="mailto:alvarez@antrop.uc.pt">alvarez@antrop.uc.pt</a>
Mariana Curto	<a href="mailto:l46256@alunos.uevora.pt">l46256@alunos.uevora.pt</a>
Mariana Gonçalves	<a href="mailto:mariana.goncalves@insa.min-saude.pt">mariana.goncalves@insa.min-saude.pt</a>
Mariana Lopes	<a href="mailto:lopesfmariana@gmail.com">lopesfmariana@gmail.com</a>
Mariana Patanita	<a href="mailto:d46100@alunos.uevora.pt">d46100@alunos.uevora.pt</a>
Mariana Sottomayor	<a href="mailto:mssottom@fc.up.pt">mssottom@fc.up.pt</a>
Marina Costa	<a href="mailto:d47455@alunos.uevora.pt">d47455@alunos.uevora.pt</a>
Marina Curto	<a href="mailto:l46256@alunos.uevora.pt">l46256@alunos.uevora.pt</a>
Marisa Carvalho	<a href="mailto:marisa-carvalho16@hotmail.com">marisa-carvalho16@hotmail.com</a>
Marisa Encarnação	<a href="mailto:marisa.encarnacao@insa.min-saude.pt">marisa.encarnacao@insa.min-saude.pt</a>
Marta Vitorino	<a href="mailto:mvitorino@ualg.pt">mvitorino@ualg.pt</a>
Miguel Marcelino	<a href="mailto:migmarcelino@gmail.com">migmarcelino@gmail.com</a>
Mónica Marques	<a href="mailto:monicasgmarqueswork@gmail.com">monicasgmarqueswork@gmail.com</a>
Natália Marques	<a href="mailto:nmarques@ualg.pt">nmarques@ualg.pt</a>
Natércia Conceição	<a href="mailto:nconcei@ualg.pt">nconcei@ualg.pt</a>
Nuno Maximiano	<a href="mailto:ngfmax1996@gmail.com">ngfmax1996@gmail.com</a>
Patrícia Beldade	<a href="mailto:pbeldade@fc.ul.pt">pbeldade@fc.ul.pt</a>
Patrícia Carrasqueira	<a href="mailto:l49414@alunos.uevora.pt">l49414@alunos.uevora.pt</a>
Patrícia Gatinho	<a href="mailto:patricia.gatinho.s@gmail.com">patricia.gatinho.s@gmail.com</a>
Patrícia Silva	<a href="mailto:patricia.silva@soquimica.pt">patricia.silva@soquimica.pt</a>
Paula Filomena Lopes	<a href="mailto:plopes@utad.pt">plopes@utad.pt</a>
Paulo de Oliveira	<a href="mailto:oliveira@uevora.pt">oliveira@uevora.pt</a>
Pedro Dias Ramos	<a href="mailto:pedro.ramos@ibmc.up.pt">pedro.ramos@ibmc.up.pt</a>
Pedro Ferreira	<a href="mailto:pg43153@alunos.uminho.pt">pg43153@alunos.uminho.pt</a>
Rafael Prazeres	<a href="mailto:r.prazeres@campus.fct.unl.pt">r.prazeres@campus.fct.unl.pt</a>
Raquel Chaves	<a href="mailto:rchaves@utad.pt">rchaves@utad.pt</a>
Raquel Ramalhosa	<a href="mailto:l48362@alunos.uevora.pt">l48362@alunos.uevora.pt</a>
Ricardo Ferraz	<a href="mailto:rikayferr@hotmail.com">rikayferr@hotmail.com</a>
Ricardo Jorge Pereira	<a href="mailto:ricper1990@gmail.com">ricper1990@gmail.com</a>
Rita Carvalho	<a href="mailto:Ritamdc.32@gmail.com">Ritamdc.32@gmail.com</a>
Rita Nobre Pires	<a href="mailto:rnpires@uevora.pt">rnpires@uevora.pt</a>
Rita Pires	<a href="mailto:rita.pires@cebal.pt">rita.pires@cebal.pt</a>
Roukaya Bem Gaied	<a href="mailto:ex16388@alunos.uevora.pt">ex16388@alunos.uevora.pt</a>
Sandra Caeiro	<a href="mailto:caeirosandra@hotmail.com">caeirosandra@hotmail.com</a>
Sandra Correia	<a href="mailto:sandraimc@uc.pt">sandraimc@uc.pt</a>
Sandra Louzada Gomes Pereira	<a href="mailto:slouzada@utad.pt">slouzada@utad.pt</a>
Sandra Viegas	<a href="mailto:sviegas@itqb.unl.pt">sviegas@itqb.unl.pt</a>

Sara Barrias	<a href="mailto:sarabarrias@hotmail.com">sarabarrias@hotmail.com</a>
Sara Freitas	<a href="mailto:siofreitas@cibio.up.pt">siofreitas@cibio.up.pt</a>
Sara Silva	<a href="mailto:saramssilva@ua.pt">saramssilva@ua.pt</a>
Sofia Alves	<a href="mailto:salves@isa.ulisboa.pt">salves@isa.ulisboa.pt</a>
Sofia Carvalho	<a href="mailto:sofia.carvalho@insa.min-saude.pt">sofia.carvalho@insa.min-saude.pt</a>
Sonya Neto	<a href="mailto:sonya@ua.pt">sonya@ua.pt</a>
Soraia Silva	<a href="mailto:soraias@ua.pt">soraias@ua.pt</a>
Susana Domingues	<a href="mailto:susanadomingues@itqb.unl.pt">susanadomingues@itqb.unl.pt</a>
Susana M. Costa	<a href="mailto:smma.costa@itqb.unl.pt">smma.costa@itqb.unl.pt</a>
Tânia Caetano	<a href="mailto:tcaetano@ua.pt">tcaetano@ua.pt</a>
Tânia Marques	<a href="mailto:trmarques@fc.ul.pt">trmarques@fc.ul.pt</a>
Tatiana Varela	<a href="mailto:a44770@ualg.pt">a44770@ualg.pt</a>
Thales Viana	<a href="mailto:thalesviana@ua.pt">thalesviana@ua.pt</a>
Tiago Lourenço	<a href="mailto:tiagolourenco@cibio.up.pt">tiagolourenco@cibio.up.pt</a>
Tony Nolan	<a href="mailto:Tony.Nolan@lstmed.ac.uk">Tony.Nolan@lstmed.ac.uk</a>
Torcato Martins	<a href="mailto:torcato@ua.pt">torcato@ua.pt</a>
Vasco Neves	<a href="mailto:vasco.mfm.neves@gmail.com">vasco.mfm.neves@gmail.com</a>
Veronica Fernandes	<a href="mailto:vfernandes@ipatimup.pt">vfernandes@ipatimup.pt</a>
Verónica Mestre	<a href="mailto:vero.mestre11@gmail.com">vero.mestre11@gmail.com</a>

---