XXXVIII JORNADAS PORTUGUESAS DE GENETICA

4-5 DE JUNHO 2013 / PORTO, PORTUGAL

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XXXVIII

JORNADAS PORTUGUESAS DE GENETICA

4-5 JUNE 2013

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GENETIC DISORDERS AND HEALTH

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16:15 – 16:30 - Genome-wide association study identifies *FUT2* as a novel genetic risk factor for Behçet's disease
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16:45 – 17:00 – *Closing Remarks*

INVITED LECTURES

INVITED LECTURE 01

Statistical proteomes: can they shake current concepts of the gene?

Manuel A. S. Santos

RNA Biology Laboratory, Department of Biology, University of Aveiro, 3810-193 Aveiro

The concept of statistical proteins was proposed in 1965 by Carl Woese in his theory of the origin of the genetic code and the translational machinery (Woese, 1965). Woese defined statistical proteins as mixtures of polypeptides whose primary structures are related to some theoretical average primary structure. In extant organisms they are synthesized through low level mistranslations of mRNA arising from tRNA misacylation, ribosome misreading, RNA editing and RNA modification. Canonical gene translation produces homogeneous mixtures of polyptpetides, but statistical proteins are heterogeneous arrays of polypetides whose cellular functions may also be heterogeneous, complicating the already complex relationship between genes and phenotypes. We have discovered that the main human fungal pathogen *Candida albicans* has a statistical proteome (Gomes et al, 2007). Its genome encodes 6202 protein genes - a similar number of genes to other fungi -, but ambiguous gene translation by the ribosome results in millions of different proteins that are not degraded by the *C. albicans* protein quality control machinery. In this fungus, there is no correlation between gene number and protein number despite the lack of alternative splicing. I will illustrate in my talk that statistical proteins are produced through global re-programming of the genetic code and have specific functions. They increase dramatically phenotypic and genetic diversity, expand adaptation capacity in changing ecological landscapes and influence virulence, biofilm formation and drug resistance. That is, statistical proteins allow *C. albicans* to produce phenotypic diversity in the absence of alternative splicing and without increasing gene number.

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Gomes A.C., Miranda I., Silva R.M., Moura G.R., Thomas B., Akoulitchev A. and Santos M.A. (2007) A genetic code alteration generates a proteome of high diversity in the human pathogen Candida albicans. *Genome Biology*, 8, pp. R206.

Acknowledgments: This work was supported by FCT/FEDER project PTDC/BIA-MIC/099826/2008 and the EU FP7 Sybaris consortium.

INVITED LECTURE 02

Strategies to uncover the drought tolerance mechanism of the biodiesel plant *Jatropha curcas* (purging nut)

Helena Sapeta[1,2], Tiago Lourenço[1,2], J. Miguel Costa[2,3], Stefan Lorenz[4], Christian Grumaz[5], Kai Sohn[4] and M. Margarida Oliveira[1,2]

[1]Genomics of Plant Stress lab, ITQB-UNL, Av. República, 2780-157 Oeiras, Portugal; [2]IBET, Quinta do Marquês, 2784-505, Oeiras, Portugal; [3]Plant Molecular Ecophysiology lab, ITQB-UNL, Av. República, 2780-157 Oeiras, Portugal;[4] Functional Genomics Lab, Fraunhofer IGB, Nobelstr. 12, 70569, Stuttgart, Germany; [5] University of Stuttgart IGVT, Noberstr. 12, 70569, Stuttgart, Germany

Jatropha curcas **is an emerging source of biodiesel due to the high quality oil content of its seeds. Historically, these plants have been spread around the world by the Portuguese during the discoveries period.** ***Jatropha curcas*** **plants are very resilient to low water availability and are amenable for cultivation in areas with poor soil quality. Because the molecular mechanisms of drought stress response of** ***J. curcas*** **are still poorly described, we started our studies by comparing the behaviour of two accessions adapted to two contrasting climates (semi-arid and wet tropical). Seedlings of these plants were submitted to drought stress by water withhold in laboratory conditions (49 days), followed by recovery (1 week), and were evaluated for several morphological and physiological parameters[1]. In parallel, samples were collected for RNA isolation and 22 cDNA libraries were constructed and sequenced using Next Generation Sequencing (NGS) technology. Sequences were annotated to the publicly available** ***Jatropha*** **genome database (http://www.kazusa.or.jp/jatropha/) and expression values were normalized using the reads/Kb/Million (RPKM) method. Seedlings of both accessions behaved similarly at morpho-physiological level, both under stress and control conditions. However, differences were clear when comparing control conditions versus drought in maximum stress. Hierarchical clustering analysis of the transcriptomic data allowed forming 3 major clades, encompassing leaf samples, root samples and a third group joining root and leaf samples under maximum stress (day 49). For day 49 we found approximately 2000 genes in roots and 2000 in leaves that are responsive to drought. These genes have been described as involved in known pathways of stress response. We have been conducting RT-qPCR for specific genes, and further confirming the RNAseq data. Moreover, we found a very high drought stress recovery capacity, with the plants showing full recovery after only 3 days of re-watering, in terms of growth, physiology and transcriptomic data. We also found interesting changes at the photosynthetic metabolism, with decrease in the chlorophyll** ***a/b*** **ratio under severe stress, supported by the transcriptomic and RT-qPCR data. At present, we are further characterizing the drought responsive system of** ***J. curcas*** **by analysing changes at the hormonal level. The most recent data will be presented.**

Acknowledgments: Quinvita for providing the seeds and Funding by FCT (PTDC/AGR-GPL/ 101435/2008).

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INVITED LECTURE 03

Conservação e melhoramento dos recursos genéticos na era da genómica

Albano Beja Pereira

CIBIO

Na ultima década o desenvolvimento tecnológico permitiu desenvolver novas técnicas de sequenciação baixando dramaticamente os custos de sequenciação de DNA. A sequenciação total do genoma de cada vez mais espécies domésticas é uma realidade que possibilita uma maior e melhor visão da variabilidade molecular destas. Nos últimos dois anos têm surgido no mercado SNPchips que permitem genotipar dezenas de milhares de mutações distribuídas ao longo do genoma. Porém, na sua maioria, estes chips testam SNPs cuja frequência alélica na maior das populações ainda não foi calculada, aumentando o risco de enviasamento na extrapolação entre populações. Nesta palestra irei demonstrar como em alternativa a re-sequenciação de alguns genes candidatos pode revelar-se uma alternativa viavel. Mais ainda, darei exemplos practicos de como a resequenciação tem ajudado a descobrir novas mutações que alteram a estruturas de proteínas importantes. Finalmente, darei exemplos acerca dos perigos e vantagens do uso de SNPchips e darei exemplos de como detectar genes/alelos que podem estar sobre selecção.

INVITED LECTURE 04

Clearance of extracellular misfolded proteins in systemic amyloidosis: experience with transthyretin

Maria João Saraiva

Instituto de Biologia Molecular e Celular – IBMC; Instituto de Ciências Biomédicas Abel Salazar – ICBAS Universidade do Porto

Increasing evidence indicates that accumulation of misfolded proteins in the form of oligomers, protofibrils or amyloid fibrils, and their consequences in triggering intracellular signaling cascades with toxic consequences represent unifying events in many of slowly progressive neurodegenerative disorders. Studies with small compounds or molecules, known to recognize and disrupt amyloidogenic structures, have proven efficient in promoting clearance of protein aggregates in experimental models of systemic and localized forms of amyloidoses. Doxycycline and EGCG were efficient in removing aggregates in pre-clinical studies in a transgenic mouse model for transthyretin (TTR) systemic amyloidosis and represent an opportunity to address mechanisms and key players in deposit removal. Extracellular chaperones, such as clusterin and metalloproteinases play an important role in this process.

ORAL COMMUNICATIONS

An evolutionary perspective on metazoan NAD salvage pathways

João Carneiro[1,2], Sara Duarte-Pereira[1], Luísa Azevedo[1,2], L. Filipe C. Castro[3], Paulo Aguiar[4], Irina S. Moreira[5], António Amorim[1,2] and Raquel M. Silva[1]

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Nicotinamide Adenine Dinucleotide (NAD) is a cofactor in redox reactions and a substrate for NAD-consuming enzymes, such as PARPs and sirtuins. The maintenance of NAD levels is crucial, thus, organisms use distinct pathways for NAD production and salvage depending on alternative precursors.

NAD salvage enzymes have gained increased attention due to their roles in aging, infection and disease and, more recently, as therapeutic targets in cancer [1]. Nicotinamide phosphoribosyltransferase (NAMPT) and Nicotinamidase (PNC) are the functional homologues in different NAD salvage pathways and, until now, NAMPT was thought to be restricted to vertebrates and both NAMPT and PNC homologues had not been found in the same lineages.

By combining molecular and structural biology with comparative and evolutionary approaches, we provide, for the first time, experimental evidence that both enzymes are expressed simultaneously in invertebrate species, identify active site residues of invertebrate enzymes and emphasize sequence and structural conservation patterns in metazoan NAMPTs and PNCs. The results indicate that both NAMPTs and PNCs can be concurrently functional in the same species, which indicates that these enzymes are not redundant after all [2].

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Screening for novel germline genes using *Drosophila melanogaster*

Paulo Navarro-Costa[1,2], Pedro Prudêncio[1,2], Jörg D. Becker[2] and Rui G. Martinho[1,2]

[1]Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; [2] IGC-Gulbenkian Institute for Science, Oeiras, Portugal.

Introduction: Sexual reproduction, the main Eukaryotic reproductive strategy, requires a germline – the immortal cell lineage responsible for gamete production. We hypothesize that Eukaryotes rely on a conserved core set of (epi)genetic determinants for segregation and development of the germline.

Objective: To identify and characterize novel evolutionarily-conserved germline genes.

Methods: We selected 52 novel genes as potentially important for germline development. These genes were selected based on two different strategies: group A (n=34) on a transcriptomics-based approach for conserved genes enriched in male germline cells of 3 divergent Eukaryote species (*Homo sapiens*, *Drosophila melanogaster* and *Arabidopsis thaliana*); group B (n=18) on a data mining strategy of publically available data sets as to identify germline genes previously associated with human reproductive disorders. A Drosophila *in vivo* RNA interference (RNAi) assay was used to functionally analyze the selected 52 genes in order to identify those associated with germline defects.

Results: We have identified a total of 18 genes (35% of the selected 52) giving distinct phenotypes in the male and female germline. These germline phenotypes include: i) loss of the germ cell lineage (n=13), ii) abnormal spermatogenesis and oogenesis (n=4), iii) abnormal meiosis (n=1). We are currently focusing our work in two of these genes that are potentially important for germline-specific chromatin remodeling and late spermatogenesis, respectively.

Conclusions: Through a Drosophila in vivo RNAi screening we have identified 18 genes with previously uncharacterized roles in the germline. On-going work on two of these candidates may ultimately reveal new mechanisms of female meiotic control and sperm biogenesis.

Genome mistranslation induces proteome aggregation and oxidative stress in zebrafish embryos

Marisa Reverendo[1*], Ana Raquel Soares[1], Manuel A. S. Santos[1]

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Protein misfolding and aggregation are associated with various human diseases and aging processes. Importantly, gene mistranslations produced by tRNA misreading in the ribosome or tRNA mischarging by aminoacyl-tRNA synthetases cause neurodegenerative diseases in humans and mice. It is not yet clear how such diseases develop and whether they are a direct consequence of proteome disruption or a result of the toxicity produced by accumulation of mistranslated proteins. To clarify this question we engineered transfer RNAs (tRNAs) to misincorporate amino acids into proteins in zebrafish embryos. The mutant proteins misfold, trigger the unfolded protein response (UPR), up-regulate the ubiquitin proteasome pathway (UPS) and down-regulate protein synthesis. Proteotoxic stress generated by these gene mistranslations affected embryo development and viability, increased the production of reactive oxygen species (ROS), accumulation of protein aggregates and mitochondrial dysfunction. Hence, proteome mutagenesis by misreading tRNAs in zebrafish embryos is a powerful methodology to destabilize the proteome for elucidating the biology of proteotoxic stres, which is a hallmark of aging and aging related diseases.

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Epigenetic regulation of Mammalian Replication Origins

Sofia Madeira[1,2], Ricardo Almeida[1,2], Rodrigo Lombraña[1], Isabel Revuelta[1], Joana Sequeira-Mendes[1] and María Gómez[1]

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The faithful duplication of every genome starts from specific chromosomal sites called origins of DNA replication (ORIs). In mammalian cells, ORI specification do not seem to rely on the DNA sequence and the regulation events behind their selection are not yet fully understood [1-2]. However, recent studies are beginning to uncover a correlation between transcription and DNA replication, since the most efficient origins of replication in both mouse and human cells are preferentially located at CpG islands promoters (CGIs) [3-5]. On the other hand, some bivalent gene promoters, which simultaneously carry active (H3k4me3) and repressive (H3k27me3) histone marks, present distinct ORI efficiencies despite of being CGIs [4]. This fact leaves the door open to the study of other factors that can influence directly or indirectly ORI selection, like the chromatin state. Regarding this, we hypothesize that some epigenetic mechanisms that influence transcription could be likewise involved in ORI regulation. In this study we tested the influence of DNA methylation, histone marks and nucleosome positioning in the regulation of ORIs. Our results indicate that DNA methylation status is unrelated to ORI activity. However, we found that histone marks associated to active transcription are highly enriched at promoters that encompass efficient ORIs. Moreover, high-resolution analyses of nucleosome positioning revealed a clear correlation between higher ORI efficiency, a well-positioned nucleosomal array, and well-located replication initiation sites in most cells of a population. In contrast, less efficient ORIs showed a more dispersed initiation points within the cell population, coincident with a less strongly-positioned nucleosomes in those regions. These results indicate that chromatin architecture could have a role in the regulation of replication initiation activity in mammalian cells.

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Temporal dynamics of Erk and Gli3 Signaling Pathways evidence parallelisms in SHH morphogen interpretation in multiple tissues of the chick embryo

Hugo Marcelino[1,2], Caroline J. Sheeba[1,2,3,4] and Raquel P. Andrade[1,2]

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All vertebrate species present a segmented articulated body, easily observed at the vertebral column level. This segmented nature can be detected quite early during embryonic development with the periodic formation of repeated segments, the somites, along the anterior-posterior embryo body axis. The strict temporal precision of somite formation from the anterior tip of the presomitic mesoderm (PSM) is underlined by the PSM molecular clock [1, 2]. A similar *hairy2*-based clock was also identified in the developing distal limb, providing temporal information to these cells [3]. Interestingly, both the PSM and limb clocks are influenced by FGF8 and SHH signaling, emanated from the tailbud/apical ectodermal ridge (AER) and the notochord/zone of polarizing activity (ZPA), respectively [4, 5]. An FGF8-dependent gradient of Erk activity is documented in the PSM [6]. Although there is a distal-proximal gradient of FGF8 signaling evidenced by its effector mkp3 expression [7], graded Erk activity has not been reported so far in the developing limb. Conversely, ZPA/SHH-dependent opposing gradients of Gli3-activator and Gli3-repressor are well characterised in the limb bud. Despite of the expression of Gli3 in the PSM [8], its activity and functional significance remain uncharacterized. Here we have assessed Erk and Gli3 pathway signaling over time upon manipulation of SHH sources in both the PSM and limb of chick embryo. The expression patterns of the clock gene *hairy2*, *fgf8*, *shh* and their downstream targets were further assessed by *in situ* hybridization. Western blot analyses were performed to evaluate the temporal responses of Erk, Akt and Gli signaling in these samples. The results obtained suggest a strong parallelism in signaling dynamics between PSM and limb bud in response to SHH deprivation. We believe this work will provide novel insights on how cells undergoing differentiation interpret multiple signaling pathways over time.

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Evolutionary conservation of the eumetazoan gene regulatory landscape

Michaela Schwaiger[1], Anna Schönauer[1], André F. Rendeiro[1], Carina Pribitzer[1], Alexandra Schauer[1], Anna Gilles[1], Johannes Schinko[1], David Fredman[1], and Ulrich Technau[1]

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Bilaterian animals differ from other metazoans in their apparent bilateral symmetry and the development of a third germ layer. Both might have facilitated the evolution of the diverse and complex bilaterian body plans. The first cnidarian genome sequence revealed that despite their morphological simplicity, this sister group to all bilaterians shares a surprisingly complex gene repertoire with vertebrates [1, 2, 3]. This raised the possibility that rather than the gene set itself it might have been the complexity of gene regulation, which differs between cnidarians and bilaterians. Here we compared the gene regulatory landscape of a cnidarian and bilaterians. To this end we profiled five epigenetic marks, RNA Polymerase 2 and p300/CBP through ChIP-seq and generated the first genome-wide prediction of chromatin states and gene regulatory elements in a non-bilaterian animal, the cnidarian *Nematostella vectensis*.

We found that the location of chromatin modifications relative to genes and distal enhancers is conserved among eumetazoans. We also show that the inhibition of CpG methylation by the presence of H3K4me3 at CpG islands is not a feature exclusive from mammalian cells, but is also ancestrally conserved. Despite *Nematostella* lacking CTCF (a factor implicated in gene regulation by distal enhancers) [4] we predicted thousands of distal enhancers and tested a portion *in vivo*, which in a large majority of cases induced expression at least partially reflecting the expression pattern of the neighboring gene.

Taken together our work shows that the genomic landscape of gene regulatory elements and associated genes is highly similar between *Nematostella* and bilaterian model organisms. This suggests that the complexity of bilaterian body plans in general did not arise through novel gene regulatory mechanisms, but it is conceivable that rather a re-wiring of a few important interactions in gene regulatory networks might have led to the evolution of new body plans and an increase in complexity in bilaterians.

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The genetic basis of *Drosophila* adaptation to viral infection with DCV

Nélson E. Martins[1], Vítor G. Faria[1], Viola Nolte[2], C. Schlötterer[2], L. Teixeira[1], S. Magalhães[3], É. Sucena[1,4]

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Experimental Evolution permits the establishment of causality between evolutionary processes and adaptation patterns. Here, we use experimental evolution of *Drosophila melanogaster* exposed to Drosophila C virus (DCV) to address the phenotypic and genotypic changes of hosts evolving in presence of parasites.

Upon exposure to the virus, Drosophila survival increased from 33% to almost 90% after 35 generations of selection. This response carried no detectable costs in fitness in the absence of infection, and was not lost after 10 generations in the absence of selection. Cross-resistance was found for other viruses, such as CrPV and FHV, but not to bacterial pathogens.

Whole genome sequencing of pooled samples of virus-selected populations and their matching controls at generation 20 uncovered two regions of significant differentiation between these groups of populations. The first corresponded to a region of 4 Mb in the 3L chromosomal arm. This region's peak of differentiation corresponds to a polymorphism in *pastrel* (pst), a gene recently associated with increased DCV resistance. The second contains a pair of significantly differentiated SNPs in the X chromosome, in genes not previously associated with virus resistance. Results with a panel of deficiencies in the 3L chromosome confirmed that deficiencies which encompass the *pst* locu show the strongest effects upon DCV infection, narrowing the region of interest to 200 kb.

Ongoing work seeks the functional validation of pst and addresses the involvement in resistance to DCV infection of other candidate genes in this 200 kb region and of the genes on the X chromosome.

Elusive functional impact of adaptive mitochondrial protein evolution in a lineage of arctic hares (genus *Lepus*) assessed from comparative mitogenomic analyses

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Mitochondria are key components of cellular metabolism, being responsible for the production of most of a cell's energy through the oxidative phosphorylation (OXPHOS) pathway. Mitochondrial DNA (mtDNA) encodes the core subunits of the proteins involved in this process. There is accumulating evidence that positive selection has an impact in mtDNA evolution but the direct role of mtDNA in adaptation is yet little understood. Hares (genus *Lepus*) constitute a privileged model to study this question, since: 1) different hare species thrive under contrasted climatic conditions that may require different metabolic rates and varying degrees of adaptation; and 2) mtDNA introgression among species of hares is common, particularly from the arctic species *Lepus timidus* into the northernmost ranges of several temperate species, and this might have influenced the evolution of these species. We analyzed the sequence of 11 complete mitogenomes (10 newly obtained) of temperate and arctic origins (including two of arctic origin found in temperate species). The analysis of patterns of codon substitutions along the reconstructed phylogeny provided statistical evidence for positive selection in the arctic mtDNA lineages. These substitutions occurred in several codons of genes coding for OXPHOS complexes, most evidently in the ATP8 gene. This raises the interesting possibility of an adaptive impact resulting from the massive introgression of an apparent functionally divergent arctic genome into temperate species. However, no predictable effect of this adaptive evolution was found on mtDNA composition, protein structure and physiochemical properties. Therefore the origin of this inferred adaptive divergence remains elusive.

The genetic structure and admixture of the NE Portuguese communities of Jewish origin: news from the autosomal markers

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Crypto-Judaism is defined as the secret adherence to Judaism while publicly professing another faith [1, 2]. In our work we aim to focus at a specific subset of their historical complex movements, namely the fate of the 16th century Iberian Jewish communities. Iberian Jews were at that time, a demographically non-negligible minority that suddenly was forced to either conversion or expulsion [3-5].

Our first results on both paternal and maternal lineages genetic markers (Y-chromosome specific and mitochondrial, respectively) concur to show that the communities scattered over the Bragança district have succeeded in maintaining a high level of genetic diversity with a clear root in the Near East. These findings are extremely surprising, as they show exactly the opposite of what is expected in isolated, small sized populations, namely a deep genetic diversity loss [4, 6].

While the absence of recombination of the uniparentally transmitted markers enables the construction of phylogenies with ease and to establish phylogeographic patterns that permit the detection of contribution of distinct maternal or paternal gene pools they don't allow the detection of population substructure nor the time and mode of admixture [7].

In this work we report preliminary results from the analysis of two types of autosomal markers: a set of forensically validated microsatellites (STRs) and a panel of ancestry informative insertion/deletion polymorphisms (InDels). We found that this population shows an absence of sub-structuration contrarily to what would be expected from data previously obtained with uniparental markers. This absence of individual or subgroup heterogeneity can be explained either by a historically recent but strong admixture process with the host population or a though slow paced homogenization for a quite extended period of time. To clarify under which of these alternative modes was the admixture predominantly taking place, the study of linked markers (namely from the X-chromosome) will be undertaken.

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Population Structure of the African Sahel inferred from Genome-Wide SNP Screening

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The recent rapid development of high-throughput genotyping technologies allows us to study the genetic structure, migrations and gene flow of populations. Here we report the first genome-wide analysis of populations from the African Sahel, the main corridor of African west-east migrations.

We carried out a genome-wide analysis of 13 populations across the Sahel Belt, summing up 173 samples of unrelated individuals, which were genotyped using an Illumina platform for 2.5 million SNPs. We discovered, through ADMIXTURE analyses, that the genetic structure of Sahel populations harbours two main components: an East-African, which is predominant in Arabs and Nubians in Sudan and Oromo people in Ethiopia (and less so for Kenyan populations of Turkana and Samburu); and a West-African, which we identified as a main feature in populations from Burkina Faso. The remaining populations showed an admixed pattern, namely the ones from the Central Sahel region (as Daza, Kanembu) and the nomadic people Fulani (from Mali).

The principal component analysis confirmed these findings. The main eigenvector separated the populations in an East-West axis. While a striking finding was Fulani position departed from other Central Sahel samples at second eigenvector, indicating an unique component in the genetic makeup of Fulani people which showed to have a big population influence from North Africa.

The admixed pattern of populations in Central Sahel testifies the role of this region as a longitudinal bidirectional corridor, which greatly influenced the genetic makeup of the entire African continent.

Detection of lead toxicity by morphological parameters and ISSR markers in *Lactuca sativa* L.

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Toxic heavy metal pollution has become an increasing worldwide environmental problem since the onset of the industry revolution. Lead (Pb) is a nonessential metal and has significant ecological relevance in many studies, since it is one of the most severe pollutants. This metal induces a broad range of toxic effects to living organisms, including morphological, physiological and biochemical alterations. Additionally, plants that grow in contaminated soils can absorb and accumulate Pb, thereby introducing the metal into the food chain by trophic transfer, including in the human diet.

In this study, plants of the cultivar "Reine de Mai" (*Lactuca sativa* L.) were grown hydroponically with Pb (0, 0.05, 0.5 and 5ppm), during a 14-days exposure period, in controlled conditions. Morphological characters including roots and shoot biomass and length were recorded in four plants of each concentration. The genotoxicity effect of Pb was evaluated in the roots of the same plants using ISSRs (Inter Simple Sequence Repeats) for DNA changes analysis.

In general, both shoot length and biomass decreased with increasing Pb concentration. Comparing with control condition, we observed an increase in the roots biomass and length for the concentration of 0.05 ppm, and a decrease in the others concentrations.

The DNA damages in plant roots were analysed with eight ISSR primers. The differences in DNA patterns were evaluated by the presence or absence of bands in treated samples, compared with the untreated. On average, the rate of polymorphism increased with the Pb concentration.

The present study shows the environmental risk of Pb on plant growth since it affected the length and biomass of the roots and shoots of this lettuce cultivar. The comparison between 'untreated' and 'treated' plants showed that ISSR analysis can be used to evaluate how the environmental pollutants modify the structure of DNA in lettuce and this method seems to be a useful tool for measuring genotoxic activity due to environmental pollutants.

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Assessment of genetic variability of *HSP101C* gene in *Triticum durum* Portuguese varieties

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Cereals are essential to human and domestic animal nutrition constituting over 50% of crop production worldwide, belonging to Triticeae the most important crops in European agriculture. Portugal has a very high deficit in cereals, importing more than 770 millions of Euros in cereals each year [1]. The demand for Durum wheat (*Triticum durum*) has been increasing considerably in the past years, being imports 28 times higher than national production in 2011 [2]. Thus, it is extremely important to characterize different Portuguese varieties genetic diversity related with plant adjustment capability to Portuguese environmental conditions.

Heat Shock Proteins (HSP) are involved in plants tolerance mechanisms to various abiotic stresses like drought, cold and particularly heat stress. This protein family shows high level of diversity, being divided into five classes: small HSP, HSP60, HSP70, HSP90 and HSP100. HSP101 belongs to the HSP100 class and besides sharing the typical HSP functions, is also known to be involved in plant fitness benefit under normal plant growth conditions. The loss of this protein results in a decrease in fruit production, days to germination and to bolting, total dry mass and number of inflorescences [3]. Therefore, taking into consideration the high phenotypic impact of HSP101 it becomes of great interest to characterize genetic variability of this gene among the Portuguese *T. durum* varieties.

In order to access intraspecific HSP101 genomic variability we sequenced *HSP101C* gene from *T.durum* Portuguese commercial cultivars and the results obtained revealed the presence of two isoforms (A and B) coding sequences in all three varieties, as previous described. Furthermore, all sequences revealed high levels of homology (~99%) with the respective HSP101C isoforms gene of *T. durum* sequences previously published in NCBI database. The comparison between A and B isoforms sequences revealed 92% homology in all varieties analyzed. These differences correspond to single nucleotide polymorphism (5%) as well as gaps although in a lower proportion (3%). When sequences of isoform A (or B) obtained from different varieties are compared only minor differences (<1%) are observed, corresponding only to single nucleotide polymorphism. Although the differences observed may affect protein final structure and function, the results obtained indicate that both isoforms of HSP101C are highly conserved among durum wheat varieties.

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Chloroplast genome variability in marginal grapevine (*Vitis vinifera* L.) cultivars from Vinhos Verdes DOC Region

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Portugal, due to its mild climate, is by excellence a wine-producing region, being characterized by a great diversity of autochthonous grapevine cultivars. The practice of viticulture, in the current territories of Vinhos Verdes DOC region, date to the III century BC. In this region, at the beginning of the XXth century, there was reference in the restricted area of same municipalities of numbers reaching around 100 cultivar names, suggesting high grapevine biodiversity richness. Currently, there are in Vinhos Verdes DOC region many minor cultivars that represent a great repository of genetic variability. These under-used cultivars are raw material that can be used for the production of new wines and for breeding programs.

Due to its conservative nature and maternal inherence, chloroplast DNA markers are a useful methodology to define phylogenetic relationships among grapevine varieties. The chloroplast microsatellites (cpSSRs), in particular, have been widely used in the genetic characterization of cultivated and wild grapevines. Iberian Peninsula has been referred as a domestication centre of grapevine due to the sharing of chloroplast haplotypes between wild and cultivated material. One chlorotype based in cpSSRs amplification, designated as A, is mentioned in the literature as typical of this region [1] [2].

The main goal of this study was to assess the genetic diversity of a group of 38 samples, greatly neglected and representative of minor Vinhos Verdes autochthonous cultivars, using three cpSSR loci, and infer their possible origin. Haplotype A was the most frequent and was found in 34 samples (89.5%). The four remaining samples presented one haplotype typical of Italian Peninsula, Near East and Middle East [1] and probably correspond to cultivars with an origin outside Vinhos Verdes DOC.

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Cytogenetic characterization of hybrids wheat containing the system msH1

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This research project is part of the international consortium Plant-KBBE-2010-HY-WHEAT involving three breeding companies: Agrasys SL (Spain), project leader, Saaten-Union Recherche SAS (France) and Saaten-Union Biotec GmbH (Germany); and two state agencies: IAS-CSIC (Spain) and UTAD (Portugal). This project seeks to validate the genetic system "male sterility H1" (msH1) recently developed by researchers of the IAS-CSIC, Cordoba, Spain, from line H1 *Hordeum chilense* L. This system is effective in producing hybrid wheat across the cytoplasmic male sterility (CMS) and fertility restoration (Rf) with the application of alternative chemicals and transgenesis. The hybrid wheats that have the system msH1 are suitable for sustainable agriculture because they require reduced inputs; present a high and stable production and tolerance to biotic and abiotic stresses. However, the msH1 system must be validated in hybrids through molecular, cytogenetic, and morphological approaches to evaluate the restoration of male fertility, production, and transmission the Rf system to the offspring by backcrosses with wheat (a reduction of H^{ch} introgression is expected).

With this study we aim to characterize cytogenetically 72 soft hybrid wheat plants that contain the msH1 system by using the Genomic In Situ Hybridization (GISH) technique with genomic DNA of *H. chilense* and rye as probes. This technique allowed the detection of chromosomes introgression involving *H. chilense* and/or of rye in 81% hybrid plants analyzed. A reduced number of plants (1%) showed the addition of an entire chromosome of *H. chilense* ($4H^{ch}$ chromosome). In 30% of wheat hybrid plants one translocation involving *H. chilense* chromosomes (T6HchS.6DL) occurred. Nevertheless two translocations T6HchS.6DL were observed only in 14% of wheat hybrid plants. Regarding the wheat-rye T1RS.1BL translocations, we identified two translocations in 9% and only one translocation in 53% of the hybrid wheat plants analyzed. Additionally, in 32% of the hybrid wheat plants that showed alien chromosomes both T6HchS.6DL and T1RS.1BL translocations were identified.

The GISH technique proved to be an accurate method to detect alien introgressions in bread wheat hybrids that have the msH1 system. The cytogenetic data must be compared with the molecular ones in order to infer the potential of the msH1 system in the production and development of hybrid wheat.

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Evidence of the existence of native *Pinus sylvestris* L. populations in Serra do Gerês by nuclear and chloroplast microsatellite markers

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The southwestern limit of *Pinus sylvestris* L. distribution lies in Portugal. At Biduissa (Bid) and Ribeira das Negras (R.N.) locals (Serra do Gerês; NW, Portugal) exist *P.sylvestris* populations that are believed to be native. Previous studies realized by our group with dominant markers supported this assumption. Molecular studies based on microsatellite (SSR) markers could confirm or disavow this hypothesis. Nuclear (nSSR) and chloroplast (cpSSR) markers are commonly used in population genetics studies and may give complementary insights into the analyzed populations since they differ in the inheritance mode and mutation rate. We analyzed five cpSSR *loci* and four nSSR *loci* with primers from Vendramin *et al.* (1996) and Soranzo *et al.* (1998) using genomic DNA from 96 individuals belonging to six populations: Bid and R.N. (Serra do Gerês); Puebla de Lillo and Montes Universales (Spain); Austria; and Germany. PCR products were analyzed by capillary electrophoresis. The nSSR *loci* revealed to be highly polymorphic, once we found 16 to 29 alleles per primer, and the cpSSRs showed 56 distinct haplotypes. High genetic diversity values, ranging from 0.668 to 1, were observed with cpSSRs. The haplotype richness (considering rarefaction) varied between 2.983 and 9.000 (n=10). Both Bid and R.N. presented the lowest Rh values, 5.274 and 2.983, respectively, as well as the highest Dst values when compared with the foreign populations. Bayesian analysis identified a relevant number of K=3 clusters. The admixture proportions clearly differentiated both Bid. and R.N. from each other, and from the remaining populations. Principal Coordinate Analyses (PCAs), based on nSSR and cpSSR data, clustered the Portuguese populations apart from the northern populations as well as from each other. The SSR results evidenced that the *P.sylvestris* populations from Serra do Gerês have different origins relatively to the foreign ones, supporting the initial proposal of being autochthonous in Portugal.

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A *C. elegans* mutant of an intellectual disability-associated gene shows behavioral deficits and GABAergic/cholinergic circuit abnormalities

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Intellectual disability (ID) is one of the most frequent and disabling neurological impairments with an estimated prevalence of 1.5-2% in Western countries. Technological advances such as the use of array comparative genomic hybridization (aCGH), and more recently, next generation sequencing, have been adopted by several genetic laboratories, in order to identify novel genetic basis of ID. This large-scale analysis originates an incredible number of novel genetic associations every year. However, these associations often lack functional validation, and for several of the identified genes, their function in the nervous system remains undisclosed. This prompted us to use a simple model, the *Caenorhabditis elegans* (*C. elegans*), to functionally validate the genetic associations and to better understand the role of a given protein in the nervous system.

So far, we have studied 25 mutant strains, that correspond to 18 orthologues of human genes previously linked to ID. One candidate, ID34, presents gross anatomical defects (multivulva, loss of vulva), developmental delay (larval arrest), embryonic lethality and decreased life span. In parallel, this strain presents motility defects (increased uncoordination) and mild chemotaxis defects, suggesting some degree of impairment of neurological function. Crossing ID34 with strains expressing GFP in specific neuronal sub-types, we found that the GABAergic network was strongly affected - abnormal neuronal positioning and migration. The cholinergic circuit also presented some defects, but not so pronounced. Consistently, animals were more sensitive than the wild-type to Pentylenetetrazol (PTZ - GABA antagonist) and to aldicarb, a cholinesterase inhibitor. We are now assessing these mutants in other behavioral paradigms to evaluate learning and memory.

Association of FTO rs9939609 polymorphism to type 2 diabetes and obesity in Portuguese women

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Background: Obesity arises from excessive fat mass accumulation and is associated to health complications and decreased life expectancy resulting from a combination of genetic, environment and lifestyle factors[1]. Excess body weight and physical inactivity is largely associated with type 2 diabetes that comprises 90% of the diabetics[2]. There are only scattered reports on the involvement of genomic factors in obesity within the Portuguese population[3,4] and none in type 2 diabetes. We aimed at evaluating the association of one obesity related SNP - FTO rs9939609, [*Fat mass and obesity associated-gene*] - to risk to obesity and type 2 diabetes in Portuguese women.

Methods: A total of 194 Portuguese female Caucasian in premenopause aged between 18-50 years old participated in this study to assesses the risk of rs9939609 (FTO) to obesity and type 2 diabetes. Ninety-five subjects were classified as case (BMI≥30 Kg/m^2) and ninety-nine as control (BMI between 18.5 and 24.9 Kg/m^2). Polymorphism characterisation via direct Sequencing. Association to obesity and type 2 diabetes was determined by odd ratios (OR) calculation with 95% of confidence interval. To determine the differences between genotype groups of rs9939609 and anthropometric traits was made an one-way analyses of variance (ANOVA) and a post hoc Bonferroni test.

Results: Significant differences in BMI between control and case group for FTO rs9939609 (P<0.05) were found, indicating higher risk to obesity in presence of both risk alleles (AA): OR=2.571 (1.048-6.308) (p=0.039). Homozygous subjects (AA) with BMI≥40 Kg/m^2, show 4 times higher risk of obesity: OR=4.044 (1.099-14.878) (p=0.035). Also, significant differences were found between rs9939609 genotype groups and fat mass (p-value=0.021) and waist circumference (p-value=0.030). Homozygous subjects (AA) with BMI≥40 Kg/m^{2}show significant differences in glycaemic status (p-value=0.013), indicating susceptibility of AA carriers to type 2 diabetes.

Conclusion: This is the first study in an adult Portuguese population showing association of FTO to obesity and susceptibility for type 2 diabetes. These results are important for the clinical management of obesity.

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Transcription factor Nrf2 Protects Against Dietary Iron−Induced Liver Injury: Is Nrf2 A Potential Genetic Modifier Of Iron Overload-Associated Liver Disease?

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Background: Excessive intestinal absorption of iron as seen in hereditary hemochromatosis causes its deposition in the parenchymal cells of various organs, which in some individuals may lead to organ failure. The liver, being the major site of iron storage, is particularly susceptible to the toxic effects of iron. Cellular injury is caused by oxygen radical−mediated damage to cellular organelles, leading to hepatocyte necrosis or apoptosis. Transcription factor Nrf2 is critical for protecting the liver against disease by activating the transcription of genes encoding detoxification/antioxidant enzymes and transporters that aid in the elimination of harmful xenobiotics. However, the activation of the Nrf2 pathway by iron overload has not been demonstrated. Aims: We aimed to determine if the Nrf2 pathway constitutes a major defense of cells and organisms against iron−induced toxicity. Results: Iron toxicity was firstly analyzed in mouse embryonic fibroblasts (MEFs) and in primary hepatocytes from wt and Nrf2−/− mice. Incubation with iron increased intracellular reactive oxygen species (ROS) and dramatically reduced cell viability in Nrf2−/− cells. Iron induced a set of cytoprotective genes only in Nrf2+/+ cells, suggesting that the Nrf2 pathway plays an important protective role against iron toxicity. To determine the significance of these findings in an animal model of iron overload, wt and Nrf2−/− mice were fed *ad libitum* standard rodent chow or iron−rich diet (2.0% carbonyl iron) for 2 weeks. Animals of both strains accumulated similar amounts of dietary iron in the liver and were able to respond by activating key regulators of iron homeostasis. Nevertheless, Nrf2−/− mice developed marked liver injury, as judged by the presence of inflammatory infiltrates, hepatocyte necrosis, extensive transferase−mediated dUTP nick−end labeling (TUNEL) staining, damaged mitochondria, hepatocytic oxidative DNA damage and increased serum alanine aminotransferase (ALT) activity. Notably, liver injury was prevented when Nrf2−/− animals on the iron−rich diet received the antioxidant mito−TEMPOL. Activation of the Nrf2 pathway by dietary iron in wild−type animals was illustrated by increased expression of two prototypical Nrf2 target genes, GSTa1 and NQO1. Conclusions: We demonstrate that Nrf2 protects mouse cells and liver against iron toxicity, which is associated with the ability of wild-type cells and organisms to activate the expression of a set of cytoprotective genes and with their higher capacity to resist iron-generated ROS. We propose that Nrf2 may be a potential modifier of iron overload-associated liver disease in humans.

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Genome-wide association study identifies *FUT2* as a novel genetic risk factor for Behçet's disease

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Background: Behçet's disease (BD) is a multisystemic immune and inflammatory disorder whose aetiology remains unclear. Genome-wide association studies (GWAS) represent the gold-standard approach to study the genetic basis of complex diseases. In order to identify novel susceptibility loci implicated in BD we have performed the first genome-wide association for BD in the Iranian population using the validated and cost-effective strategy of DNA pooling [1].

Methods: 292 cases and 294 age- and gender-matched controls were used in the first phase of the GWAS. Two pools of cases and two pools of controls were genotyped in quadruplicate using the Affymetrix Human SNP 6.0 arrays which assess 906,600 SNPs. The SNPs were ranked by relative allele score difference between cases and controls (RAS_{diff}) and the 56 SNPs with higher RAS_{diff} were selected for technical validation through individually genotyping. The validated SNPs were further tested for association in an independent Iranian dataset of 684 cases and 532 controls. Fine-mapping of *FUT2* was performed in the Iranian discovery and replication samples combined and was also explored in a Turkish GWAS data.

Results 46 out of 50 SNPs (92%) were technically validated ($P < 0.05$ with individual genotyping in the discovery dataset). Four of these SNPs (rs9266406 and rs6910516 in the *HLA-B* locus, rs7528842 upstream of *DPH5* and rs632111 in the 3´UTR of *FUT2*) were also associated in the replication dataset. Fine-mapping of *FUT2* together with meta-analysis with the results of a Turkish GWAS [2] showed a strong association with BD of the rs602662 coding variant in *FUT2* ($P_{meta\text{-}analysis}$ = 2.87E-08, OR_G[95% CI] = 0.78 [0.72-0.85]).

Discussion rs602662 is in strong LD (r^2=0.84 in CEU HapMap) with rs601338, a functional *FUT2* polymorphism which determines the ABO antigens secretor status in body secretions and on the intestinal mucosa [3]. Approximately 20% of Caucasians are non-secretors, a phenotype associated with dysbiosis, susceptibility to infections and auto-immune diseases. This novel *FUT2* association may constitute the missing link between genes and environment in BD susceptibility and warrants further investigation.

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Exome-Sequencing in familial thyroid carcinoma: genetic and functional characterization

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Thyroid tumors are the most frequent endocrine neoplasia [1]. These tumours may occur sporadically or in a familial context, as is the case of familial nonmedullary thyroid cancer (FNMTC) [2]. FNMTC is a familial form of thyroid cancer whose genetic cause is still unknown [3,4]

Next-Generation Sequencing (NGS) technologies are enabling a new way to explore disease-causing mutations, namely in the field of oncobiology research [5,6].

The main goal of this work was to expand the genetic profile of FNMTC through NGS of different samples of one family displaying clinicopathological features suggestive of hereditary disease.

Novel germline variants as well as somatic mutations occurring *de novo* in the tumour tissue were confirmed by PCR (Polimerase Chain Reaction) and Sanger Sequencing.

From 35 candidate germline alterations identified by NGS, 1 nonsense mutation in *PRAMEF1* gene (p.L105*) was shared among affected individuals of the same family. Despite being present in a control population, distribution of this variant was significantly different from Hardy-Weinberg equilibrium, no homozygote was found in the control group. This can be taken to indicate that this variant is deleterious when in homozygosity.

In terms of somatic mutations, out of 103 candidate changes identified by NGS, 88 (85%) proved to be false-positive and 15 variants were confirmed by Sanger Sequencing. Of these, 8 were evaluated as they were classified as probably and possibly damaging by the program Polyphen-2. In this analysis loss of heterozygosity in the gene *GRID2IP* was observed. Following-up on this observation, *in vitro* experiments were conducted using an siRNA in order to reduce *GRID2IP* gene expression, mimicking inactivation observed in the tumor. The reduction in *GRID2IP* gene expression did not result in significant changes to the proliferation rate or apoptosis. Nevertheless, *GRID2IP* knock down led to an observable decrease in actin labeling at the plasma membrane, which reinforces the role of this protein in anchoring the actin cytoskeleton to the membrane and may indicate that *GRID2IP* is a tumor suppressor gene in follicular thyroid carcinoma (FTC).

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POSTERS

POSTER 01

Captive Iberian lynx (*Lynx pardinus*) as reservoirs of antimicrobial resistant enterococci.

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The Iberian lynx (*Lynx pardinus*) is the most endangered feline species and a captive breeding and conservation programme is in force. In studying antimicrobial resistance and ecology of populations of bacteria like the enterococci, it is valuable to analyse the prevalence and genetic basis of antimicrobial resistance in faecal enterococci recovered from animals bred in captivity compared to those from wildlife, domesticated animals and humans, for example.

Faecal samples were collected from captive animals originating from the breeding facilities in the Centre of Analysis and Diagnosis of Wildlife (CAD) in Doñana National Park, South Spain. In total 98 faecal samples from Iberian lynx were seeded in Slanetz-Bartley medium. Susceptibility to 11 antibiotics (vancomycin, teicoplanin, ampicillin, chloramphenicol, tetracycline, erythromycin, quinupristin–dalfopristin, ciprofloxacin, streptomycin, gentamicin, and kanamycin) was tested in disk diffusion agar assays. High-level resistance (HLR) to aminoglycosides was evaluated. Gelatinase and haemolysin production by enterococci isolates was also evaluated. The genetic basis of antibiotic resistance and the presence of genes encoding virulence factors were studied by PCR.

A total of 96 enterococci isolates from the captive Iberian lynx specimens were obtained. *E. hirae* was the most prevalent species (35 isolates), followed by *E. faecalis* (30 isolates), *E. faecium* (27 isolates), and *E. durans* (4 isolates). High percentages of isolates showed tetracycline, erythromycin and high level-kanamycin resistance (41%, 26%, and 19% respectively). Moreover, the *tet*(M) and/or *tet*(L), *erm*(B), *aac*(6')-Ie-*aph*(2")-Ia, *ant*(6)-Ia, or *aph*(3')-IIIa genes were detected among the resistant enterococci.

Here we found evidence for resistance genes in enterococci from captive specimens of Iberian lynx. The same genes that encode the antimicrobial resistance genes found in this study are found in bacteria from environmental and human sources. This indicates that bacteria and their resistance genes circulate freely between animal, environment and human ecosystems. Furthermore, the susceptibility of this endangered species to bacterial infection may be affected by the presence of virulence and resistance genes.

Evaluation of candidate reference genes for *Pinus pinaster* Ait. and *Pinus sylvestris* L. for qPCR analyses

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For the analysis of quantitative real time polymerase chain reaction (qRT-PCR) data it is required the normalization against a housekeeping or endogenous gene with an uniform expression for all tested conditions. No single housekeeping gene is universal for all experiments. Thus, a systematic characterization of different candidate reference genes for defined conditions to be tested is mandatory. In order to study the expression levels of different genes related to the process of wood formation in three tissues (needle, phloem and xylem) of *Pinus pinaster* Ait. and *Pinus sylvestris* L., we evaluated six candidate genes, selected among those commonly used as endogenous reference in conifers: *Elongation factor 1-α* (from *P. pinaster*); *Elongation factor 1- α* (from *Pinus taeda*); α-Tubulin; actin; SAND family protein (from *Arabidopsis thaliana*) and the 18S ribosomal RNA gene.

The total RNA from needle, xylem and phloem tissues was extracted by an optimized CTAB-based protocol, on a total of 18 samples. For qPCR experiments we used 10^{-1} dilutions of each cDNA sample. After analyses of the qPCR data, we verified that the *Elongation factor 1-alpha* (from *P. pinaster*) was the most suitable for further quantitative studies of gene expression in *P. pinaster* and *P. sylvestris* because it showed the lowest variation in its expression level when all the samples were analyzed together. Based on the Ct values, this candidate reference gene showed the lowest accumulated standard deviation for both species under study (around 0.5). This gene also showed the best efficiency value (109%). Despite the widely reported inadequate use of a single reference gene per qPCR analysis, among the group of candidate genes tested here, the *Elongation factor 1-alpha* (from *P. pinaster*) seems to be the most suitable reference gene for the tested conditions.

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IRAP and REMAP fingerprinting in hexaploid tritordeum (X *Tritordeum* Ascherson et Graebner) and respective parents

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Allopolyploidy constitutes an evolutionary and a revolutionary event because it implies the hybridization of two or more genomes on a single nucleus and chromosome duplication. Rapid changes at the sequence and chromosomal levels occur during the allopolyploidization. Retrotransposon (RTN)-based markers such as IRAP (Inter-Retrotransposon Amplified Polymorphism) and REMAP (Retrotransposon-Microsatellite Amplified Polymorphism) have been considered useful for DNA fingerprinting and for the detection of rapid inducing genomic restructuration in allopolyploids, among other applications. As far as we known, RTN-based markers never were been used in the hexaploid tritordeum (X *Tritordeum* Ascherson et Graebner; 2n=6x=42; $H^{ch}H^{ch}AABB$) for those purposes. This synthetic amphiploid resulted from crosses between the wild barley *Hordeum chilense* L. (2n=2x=14; $H^{ch}H^{ch}$) and durum wheat *Triticum turgidum* L. (2n=4x=28; AABB).

In this study, we intend to use DNA fingerprinting based on IRAP and REMAP markers in two tritordeum lines, HT22 and HT27, produced from crosses between *H. chilense* lines H1 and H7 respectively (female parents), and the durum wheat T81 line (male parent). Besides, we also intend to compare the molecular patterns produced in all tritordeum individuals and respective parents in order to infer about probable genomic restructuration that might occur during the process of allopolyploidization.

After testing several LTR and/or SSR primers, we achieved 6 IRAP and 7 REMAP primer combinations that produced polymorphic patterns in both HT22 and HT27 lines, and respective parents.

The IRAP polymorphism was higher (86 %) than REMAP (82 %). Most of the polymorphic bands were common among the tritordeum individuals and their male parent (T81). This result could be explained by the fact that durum wheat contributes with more genomes than *H. chilense* parent for the genomic constitution of the hexaploid tritordeum ($H^{ch}H^{ch}AABB$). Additionally, unique IRAP and REMAP bands were detected only in the tritordeuns (being absent in their parents) suggesting the amplification of newly RTN sequences. Conversely, we also found out bands shared by the parents that were absent in the tritordeuns, revealing the elimination of RTN sequences during allopolyploidization.

In conclusion, IRAP and REMAP markers proved to be useful for DNA fingerprinting of allopolyploids and respective parents, as well as for the detection of rapid genomic changes induced by allopolyploidization.

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Molecular Genetics Detection of Cyanobacteria and Cyanotoxins Worldwide

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Cyanobacteria are widespread and found in a diverse range of ecological niches including terrestrial and aquatic environments. The main importance in studying them is due to the production of dense blooms and the release of toxic compounds designated cyanotoxins, which can damage the aquatic environment and endanger the life of both animals and humans that rely of those aquatic ecosystems. In this sense the purpose of this study was to detect through PCR analyses which genera of cyanobacteria are found in water samples obtained worldwide (Europe, Asia, America and Africa). Also a screening of the major cyanotoxins was performed (microcystins, cylindrospermopsin, saxitoxin and anatoxin-a) using previously described primers.

The results showed the presence of the species *Cylindrospermopsis raciborskii* in Costa Rica, being the first report of this species in that country, and also in South Africa where previous reports have indicated the presence of this cyanobacterium species. Also *Microcystis aeruginosa*, the most common cyanobacteria, was found in Mali, South Africa and Brazil. The species *Planktothrix* was found in South Africa, Brazil and Chile. Cyanotoxin microcystin (*mcyA*) was found in Mali, Costa Rica and Brazil. Furthermore, cylindrospermopsin (*pks*) was detected in South Africa.

The PCR technique applied in this work proved to be faster and more reliable than the other methods that determine cyanobacteria and cyanotoxins presence due to its specify to detect strains of cyanobacteria as well as cyanotoxins, revealing to be a valuable tool in the monitoring of water quality.

In the future, we plan to continue this kind of work to evaluate the risk management associated with the arising of blooms favored by global warming.

Keywords Cyanobacteria . Cyanotoxin . PCR . Risk Management.

Systems biology of *Candida albicans* mistranslation

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The genetic code is not universal. Alterations to its standard form have been discovered in both prokaryotes and eukaryotes and demolished the dogma of an immutable code. For instance, several *Candida* species translate the standard leucine CUG codon as serine. In the case of the human pathogen *Candida albicans*, a serine tRNA ($tRNA_{CAG}^{Ser}$) incorporates *in vivo* 97% of serine and 3% of leucine in proteins at CUG sites. Such ambiguity is flexible and the level of leucine incorporation increases significantly in response to environmental stress. To elucidate the function of such ambiguity and clarify whether the identity of the CUG codon could be reverted from serine back to leucine, we have developed a forced evolution strategy to increase leucine incorporation at CUGs and a fluorescent reporter system to monitor such incorporation *in vivo*. Leucine misincorporation increased from 3% up to nearly 100%, reverting CUG identity from serine back to leucine. Growth assays showed that increasing leucine incorporation produced impressive arrays of phenotypes of high adaptive potential. In particular, strains with high levels of leucine misincorporation exhibited novel phenotypes and high level of tolerance to antifungals. Whole genome re-sequencing revealed that increasing levels of leucine incorporation were associated with accumulation of single nucleotide polymorphisms (SNPs) and loss of heterozygozity (LOH) in the higher misincorporating strains. SNPs accumulated preferentially in genes involved in cell adhesion, filamentous growth and biofilm formation. Global proteome, transcriptome and metabolome analysis of the mutant strains is being carried out to fully understand at the systems biology level the impact of genetic code ambiguity in *C. albicans* biology.

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High intra- and low inter-populational levels of genetic variation in the coastal *Limonium* diploid and tetraploid species of Portugal

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The *Limonium ovalifolium* complex (sea lavenders) includes three morphologically-similar diploid sexual species (2*n*=2x=16): *L.ovalifolium* (Poir.) O. Kuntze, *L. nydeggeri* Erben and *L. lanceolatum* [Hoffmanns & Link] Franco [2]; [4]. In addition natural hybrids between diploid *L. ovalifolium* species and the putative tetraploid apomict (2*n*=4x=32, 35, 36) *L. multiflorum* Erben also occur [1]. All these species have a patchy distribution across southern Atlantic Iberia, two of which are found on limestone sea cliffs while the third inhabits sandy salt marshes [3]; [1]; [2]. The aim of this study is to quantify gene flow and genetic differentiation between these taxa, considering that apomixis is often correlated with hybridization between sexual species. Canonical discriminant analyses conducted in sixty-individuals using ten diagnostic metric characters revealed clear morphological differentiation between the three sexual species. High within population variation (using the chloroplast *trnL* intron, and the *trnL-trnF* intergenic spacer) was measured in ten individuals of nine populations of *L. ovalifolium*. In contrast, low levels of genetic differentiation between populations and low F_{st} values imply that gene flow between populations via pollen is occurring. We hypothesize that the present geographic distribution is the result of long-term dispersal along the Atlantic Portuguese coast, followed by selection for particular genotypes and reproductive forms in specific environmental (substrate) conditions.

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Overexpression of wild-type, expanded and catalitically inert ataxin3 in neuroblastoma cell lines: effects on cytoskeleton organization and cell adhesion

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Machado-Joseph Disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disorder. It is caused by an expanded CAG repeat in the coding region of the ataxin-3 gene (14q32.1) which encodes for a protein with deubiquitylase activity called ataxin-3 (atx3) [1,2]. For this reason MJD belongs to the group of polyglutamine (polyQ) associated diseases.

The goal of this work was to assess the effects of mutant ataxin-3 in neuronal cell cytoskeleton organization. For that purpose, and by viral transduction, we created four different SH-S5Y5 cell lines, each one of them with distinct degrees of wild-type (14Q) and expanded (83Q) atx3 overexpression; additionally, we created a dominant-negative cell line, bearing a mutation on catalytic cysteine 14 of ATXN3. We proceeded to the characterization of these cell lines in basal conditions and after treatment with retinoic acid, which is known to induce neuronal differentiation in SH-S5Y5 cells. Cell death was analyzed by flow cytometry and migration features were approached by the scratch wound assay. Also immunocytochemistry, labeling for specific cytoskeletal components - vimentin, tubulin, actin - gave us significant clues on the cells' phenotype.

Our findings reveal cytoskeletal disorganization in an overexpression context, with distinct levels of phenotype severity between cell lines. We have also observed migration and differentiation abnormalities, supported also by the results obtained by quantitative RT-PCR of a panel of cytoskeleton regulators. The genes that show significant altered expression are involved mainly in cell projections, motility, cell cycle and G-protein signaling. These observations partially overlap with those made in cells lacking ATXN3, reinforcing the hypothesis of a partial loss of function of ataxin-3 in MJD.

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POSTER 08

Loss of function of the deubiquitylase Ataxin-3 reduces alpha-5 integrin subunit levels and leads to an abnormal neuronal differentiation process

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Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3, is a late onset neurodegenerative disease caused by an expanded polyglutamine tract within the protein ataxin-3 (ATXN3). This protein is known to bind ubiquitylated substrates and act as a deubiquitylase *in vitro*, and has been proposed to be involved in transcription regulation. Additionally, functional analyses in different cell and animal models support its relevance for protein quality control pathways, cellular stress responses, and cytoskeleton and cell adhesion regulation.

In this work we explored the normal function of ATXN3 in neuronal cells by causing stable depletion of the protein in SH-SY5Y cells using lentiviral transduction of shRNA. This caused an abnormal differentiation of SH-SY5Y cells through the modulation of alpha-5 integrin subunit levels, which in turn lead to a deregulation of the ERK and PI3K/Akt pathways. Upon retinoic acid exposure, which normally leads to differentiation into neuron-like cells, shATXN3-silenced cells presented reduced levels of many neuronal markers, maintaining expression of non-differentiated cell markers as well as an increased proliferating activity. In addition, these cells exhibited marked morphological changes such as a rounder shape, associated with decreased adhesion, and an increased number of filopodia, in contrast with a reduced number of longer processes. Levels of the alpha-5 integrin subunit, a known interactor of ataxin-3, were decreased at the protein but not mRNA level, and this was reflected in a significantly reduced adhesion to fibronectin and in reduced activation of molecules such as Rho, Rac1 and the PI3K and Erk/Akt pathways. This cellular phenotype was accompanied by an altered structure of the actin cytoskeleton and perturbation of several cytoskeletal regulators at the transcriptional and post-translational level. Cell death was also increased in ATXN3-depleted cultures.

Interestingly, very similar morphological and cytoskeletal alterations were observed in SH-SY5Y cells overexpressing ATXN3 with a mutation in its catalytic site (C14A) that abolishes the deubiquitylase activity.

In summary, we show that the absence of ataxin-3 leads to an abnormal differentiation and an overt cytoskeletal phenotype in neuronal cells. Since neurons are highly dependent on a well-structured cytoskeletal network, depletion of ATXN3 may have several downstream consequences in terms of neuronal function, which we will explore further. Our results suggest that ataxin-3 plays a role in the organization of the cytoskeleton, namely during neuronal differentiation, that is likely related to specific targets of its DUB activity.

Evolution of *ataxin-3* paralogues and transcriptional pattern of *ATXN3L1* in humans

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Ataxin-3 **gene (*ATXN3*; 14q32.1) encodes a ubiquitously expressed deubiquitinating enzyme, conserved throughout eukaryotes, with homologues among metazoans and found also in plants and protozoans [1]. In humans, *ATXN3* may expand and encode an abnormally long polyglutamine stretch, polyQ, responsible for the most common dominant ataxia worldwide: Machado-Joseph disease (MJD/SCA3) [2]. The expanded protein gains toxic properties but the normal ataxin-3 seems also to influence the pathogenesis [3]. *ATXN3* has two paralogues, originated by transposition events: *ataxin-3 like* (*ATXN3L;* Xp22.2) and *LOC100132280* (8q23.2), here named *ATXN3L1* and *ATXN3L2*, respectively. Both paralogues remain poorly studied, but a recent *in vitro* study has shown the ability of ATXN3L1 to cleave ubiquitin substrates, with its protease domain significantly more efficient than the ATXN3 domain [4].**

Our aims are (1) to study the mechanisms that led to the human-specific $(CAG)_n$ expansion in the parental *ATXN3*, but not in other lineages or paralogues; (2) to estimate the onset of the retrotransposition events; (3) to compare the rates of evolution and selective constraints for all three genes in the primate lineage; and (4) to assess the mRNA expression pattern of the retrocopy that conserved an intact ORF (*ATXN3L1*) in human tissues. We constructed Neighbor-Joining phylogenetic trees for *ATXN3*, *ATXN3L1* and *ATXN3L2* based on highly homologous sequences retrieved from several mammalian genomes available in public databases. We further completed poorly annotated primate genomes with our own sequencing data. Maximum likelihood estimates of ω (d_N/d_S) were performed by using the *codeml* program from PAML. We have found a *ATXN3L1* CAG repeat poorly polymorphic and highly interrupted across the primate lineage, whereas a pure $(CAG)_n$ was observed in *ATXN3L2* of most species, with humans and chimpanzees showing a polymorphic $(CGGCAG)_n$. Instability encompassing this hexanucleotide may have resulted from a CAG to CGG mutation occurred after the split between *Gorilla* and *Pan*. Phylogenetic results suggest that *ATXN3L1* and *ATXN3L2* arose by two independent retrotransposition events, in Haplorhini (~63 MYA), and before the Platyrrhini-Catarrhini split (~43MYA), respectively. *ATXN3L1* has been under selective constraints throughout primate evolution, suggesting a functional relevance for this retrogene, whereas *ATXN3L2* gained premature stop codons that likely turned it into a processed pseudogene. In fact, we confirmed by Reverse Transcriptase PCR, that *ATXN3L1* is transcriptionally-active, with mRNA expression in testis, placenta, brain, spleen, and thymus. Further studies will be done to confirm the presence of endogenous protein *in vivo* and to better understand the functional diversification of ATXN3L1.

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Localization of SSR sequences in chromosomes of rye and triticale using ND-FISH

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The production of amphiploids and newly formed allopolyploids induce alterations in sequences and/or chromosome rearrangements.

Over the years, Fluorescence *In situ* Hybridization (FISH) has proved to be a successful technique for mapping of repetitive DNA sequences, single copy sequences, and detection of chromosome rearrangements in metaphase chromosomes of allopolyploids or amphiploids, contributing for structural, comparative, functional and evolutionary cytogenetic studies. The Non-Denaturing FISH (ND-FISH) constitutes one variant of FISH which do not require chromosome denaturing and allows a fast achievement of results (on a single day), being more cost-effective than conventional FISH.

With this study, we aimed to analyse the physical distribution of 16 microsatellite (Simple Sequence Repeat – SSR) probes in mitotic chromosome spreads from rye (2n=2x=14; RR) and the amphiploid triticale (2n=6x=42; AABBRR) by ND-FISH, in order to compare the hybridization patterns on chromosomes from the R genome in both plant materials. For the identification of the satellited chromosomes by the detection of the rDNA *loci*, we reprobed all chromosome spreads with the probe 45S of ribosomal DNA (rDNA) (pTa71) using ND-FISH. For the discrimination of the parental genomes in triticale, we used Genomic *In situ* Hybridization (GISH) performed with the genomic probe of rye and blocking DNA of durum wheat (2n=4x=28; AABB).

Out of the 16 SSR probes tested here, only the SSR sequence $(AAC)_5$ presented hybridization in the rye chromosome spreads, showing intense hybridization signals on the centromeric, pericentromeric and telomeric regions. After using the same SSR sequence on triticale, we verified hybridization signals of $(AAC)_5$ with a stronger intensity in the wheat chromosomes, and a lower intensity in the R chromosomes. However, the centromeric, pericentromeric and telomeric locations were maintained in the chromosomes of all genomes (A, B and R). The difference of intensities among chromosomes from the R genome in rye and triticale could be due to a reduction in the number of repetitions of the sequence $(AAC)_5$ during the production of the amphiploid.

A rye karyotype for the $(AAC)_5$ SSR sequence was constructed based on a previous ideogram and the estimation of the chromosome arms ratio. The cytogenetic results achieved here, differed in part from those reported by other authors in rye with conventional FISH. This study revealed that ND-FISH could be useful for comparative analyses of physical distribution of SSR sequences (or others) among parental species and respective interspecific hybrids or amphiploids.

The inhibition of the *S-RNase* cytoxicity in Rosaceae: the *Prunus* 'one *S*-pollen gene' model and the Pyrinae 'non-self recognition by multiple *S*-pollen factors' model

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Self-incompatibility (SI) is a major genetic barrier to self-fertilisation in which the female reproductive cells recognize pollen from genetically related and non-related individuals, and reject the former. In gametophytic SI (GSI) the pollen is rejected when it expresses a specificity that matches either of those expressed in the style and is thought to have evolved once before the split of the Asteridae and Rosidae. The *S*-pistil gene product in Rosaceae, Rubiaceae, Solanaceae and Plantaginaceae is an extracellular ribonuclease, called *S-RNase*. Therefore, similarities are expected when comparing the GSI players in these plant families. In *Prunus* (tribe Amygdaloideae of Rosaceae), the self-incompatibility *S*-pollen is a single F-box gene that presents the expected evolutionary signatures for the *S*-pollen, namely pollen expression only, linkage with the *S-RNase* gene, high levels of synonymous and non-synonymous diversity and positively selected amino acid sites that account for the many specificities present in natural populations. However, clusters of F-box genes have been described in *Malus* and *Pyrus* (subtribe Pyrinae of Rosaceae) that were called *SFBB*s, *Petunia* and *Nicotiana* (Solanaceae), which are expressed in pollen only, are linked to the *S-RNase* gene and, although polymorphic, present levels of diversity 10 times lower than the *S-RNase* gene. Even so, they have been suggested as putative *S*-pollen genes, in a system of non-self recognition by multiple factors. Subsets of allelic products of the different *SFBB* genes interact with non-self S-RNases, marking them for degradation, and allowing compatible pollinations. We performed a detailed characterization of *SFBB* genes in Sorbus aucuparia (Pyrinae) to address three predictions of the non-self recognition by multiple factors model. As predicted, the number of SFBB genes was large to account for the many S-RNase specificities. Secondly, like the *S-RNase* gene, the *SFBB* genes were old. Thirdly, amino acids under positive selection - those that could be involved in specificity determination - were identified when intra-haplotype SFBB genes were analysed using codon models. Overall, our findings support the non-self recognition by multiple factors model.

Bisphenol A induces single point mutations in *C. elegans* germline

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Bisphenol A (BPA) is a worldwide highly produced chemical used in several consumer products such as food and drink containers leading to generalized human exposure. Although BPA toxicity remains highly controversial particularly at low dosages, several effects have been associated to BPA [1]. In *Caenorhabditis elegans* it was previously shown that exposure to BPA concentrations of 0.5 and 1.0 mM results in disruption of double-strand break repair (DSBR) mechanism during meiosis [2]. Using these same growth conditions and single worm random amplified polymorphic DNA PCR (RAPD) with a single primer (OPB-17), we found that genomic alterations are detected in the progeny of exposed individuals. Alterations in the RAPD stable control banding profile were not detected in the exposed progenitors but were present in 15% of individuals descendent from exposed progenitors grown continuously in control conditions since egg stage. Both loss of bands and the occurrence of new bands in relation to the control RAPD banding profile were identified. To further characterize BPA induced alterations, the 2 most frequent new bands (~1250bp and ~750bp) were excised from gel, purified, cloned and sequenced from three individuals or two individuals for the ~1250bp ~750bp fragments, respectively. Sequencing results revealed the presence of 6 unrelated sequences, 2 corresponding to the ~1250bp band with 1169bp and 1229bp and 4 to the ~750bp band with 741bp, 746bp, 751bp and 782bp. Importantly, in several cases the same exact sequence was found in more than one individual. All 6 sequences were analyzed and compared with GenBank and *C. elegans* genome browser data and 741bp, 746bp, 751bp, 782bp, 1169bp and 1229bp sequences were mapped to chromosomes V, X, III, V, V and II, respectively. In comparison to the published *C. elegans* genome sequence, pairwise analysis revealed that all 6 sequences have nucleotide polymorphisms exclusively at the primer binding sites. These alterations, identified as insertions of 1 to 4bp corresponding to the 5' end of the primer in 5 of the 6 sequences or a base pair substitution on both primer-biding sites. Taken together, our results demonstrate that BPA induces germline point mutations transmitted to the progeny in *C. elegans*, that are recurrent and not chromosome specific, consisting in a clear evidence of BPA genotoxicity.

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IS EUGENOL GENOTOXIC?

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Eugenol (CAS number: 97-53-0) is a natural alkylbenzene worldwide used in dentistry practice [1], due to his anesthetic properties, as food flavoring [2] and in cosmetic industry [3]. The aim of this study was to determine the genotoxics effects of this compound in *Drosophila melanogaster* in vivo and in human lymphocytes in vitro.

Our first aim was accomplished by exposing *Drosophila* to 9 different eugenol concentrations (0.01%, 0.03%, 0.06%, 0.1%, 0.3%, 0.6%, 1%, 3% and 6% in PBS) in a chronic way. Our results showed that the top 6 concentrations tested were to toxic, since all the *Drosophila* in those treatments died. Despite there was some survivors in the minors concentrations, they didn't had progeny, suggesting that eugenol may affect the germinal pathway.

To achieve our second goal we treated the lymphocytes, during the alkaline single cell gel electrophoresis assay (SCGE), with the 3 minors concentrations tested in *Drosophila* and two periods of exposure (15 and 30 minutes). DNA damage in lymphocytes was quantified in arbitrary units (A. U.) and a two way ANOVA test was performed. The SCGE assay detects multiple kinds of DNA damage [4] so combined with the fact of being an easy and with small expenses [5] associated toxicity evaluation test made it the chosen test to examine eugenol's genotoxicity properties.

Through the results obtained it was visible that both concentration and time of exposure affect the amount of damages, reaching, in the top concentration, the 200 A.U. showing that, unlike the FDA opinion (this product is considered safe [6]), eugenol, at least in our test conditions induces genotoxic damages in humans. These results suggest the need of further and more incisive studies about the true adverse effects of a product so extensively used as eugenol.

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Does the genetic suppression of the Nrf2 signalling pathway lead to the development of liver injury in a mouse model of spontaneous iron overload (HFE knock-out)?

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Background: HFE-associated hereditary hemochromatosis (HH) is the most common genetic disorder of iron overload among Caucasians. If untreated, it can lead to total body iron overload with secondary tissue damage in several organs attributed to oxidative stress. Most HH patients are homozygous for the C282Y mutation in the HFE gene. However, symptoms are highly variable. The low penetrance of the C282Y mutation indicates that C282Y homozigosity is a necessary but not sufficient factor in causing the disease[1]. We hypothesized that resistance to oxidative stress may be a modifier of disease progression in HFE−HH. Transcription factor Nrf2 plays a key role in adaptation to oxidative stress by regulating the induction of antioxidant/cytoprotective genes[2]. Previously, we observed that $Nrf2^{-/-}$ mice develop marked liver injury when fed a diet containing an excessive amount of iron (T.L. Duarte, manuscript in preparation). Aims: To evaluate if the genetic suppression of Nrf2 predisposes the $HFE^{-/-}$ mouse, a model of HH where iron deposition occurs spontaneously and gradually with aging, to the development of liver damage. Results: Female C57BL/6 (B6), $HFE^{-/-}$, $Nrf2^{-/-}$ and double knock-out ($HFE/Nrf2^{-/-}$) mice (n=4-8) were sacrificed at the age of 6 or 12 months. Hepatic non-heme iron, serum iron and transferrin saturation were significantly elevated in the $HFE^{-/-}$ and $HFE/Nrf2^{-/-}$ groups to a similar extent. Nevertheless, the pattern of hepatic iron distribution was different. Whilst in $HFE^{-/-}$ mice iron accumulated within the liver parenchyma, in $HFE/Nrf2^{-/-}$ animals iron was retained predominantly within aggregates of heavily iron laden sinusoidal macrophages ('siderotic nodules'). We found no differences in body weight, relative liver weight, serum transaminase activity, hepatic glutathione and antioxidant enzymes activity of 6 months old animals. At 12 months old, there were also no differences in hepatic glutathione or antioxidant enzymes activity. Nevertheless, we observed a significant loss of body weight and a small but significant increase in serum AST activity in $HFE/Nrf2^{-/-}$ animals. In some individuals we observed the deposition of collagen fibers surrounding the siderotic nodules, suggestive of early fibrinogenesis. Conclusions: Nrf2 modulated the distribution of hepatic iron, as evidenced by the formation of siderotic nodules in $HFE/Nrf2^{-/-}$ mice. Whilst there was apparently no liver injury in these animals at 6 months old, our data suggested that hepatic fibrosis may develop in older animals.

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Age-at-onset variability in FAP ATTRV30M: the triad of TTR, gender and genetic modifiers

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Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis. A wide variability in age-at-onset (AO) has been uncovered, including among Portuguese patients [17-82 yrs]. Early (≤40) and late-onset (≥50) cases are not separate entities, often coexisting in the same family, with offspring showing anticipation - a much earlier AO than their affected parent. Previous studies with Portuguese, Swedish and Japanese families have shown the presence of a marked anticipation. Mechanisms involved in anticipation in FAP, however, remain elusive. All members of the families, where AO variability was perceived, are carriers of the ATTRV30M mutation; therefore genetic modifiers might be responsible for the observed anticipation.

Our aims were: 1) to study anticipation in a larger number of kindreds than assessed before and to gain more insight into parent-of-origin effects; 2) to search for genetic modifiers, within or closely linked to the TTR locus, that may in part explain the observed AO variability; 3) to study candidate-genes, unlinked to TTR, that may also be influencing AO.

From the UCP-registry, we analyzed 926 parent-offspring pairs with well-established AO to study anticipation. So far, we ascertained 232 DNA samples of around 80 FAP families affected with the ATTRV30M mutation. We also analyzed 63 control trios.

Haplotype analysis is underway, using intragenic SNPs for extended haplotypes. Fourteen tagging SNPs were selected, with a minor allele frequency (MAF) of 0.1% and covering 60 Kb around the TTR locus.

We selected two candidate-genes (*APCS* and *RBP4*), which were assessed in a previous study in Portuguese patients[1], although variation between generations was not taken into account. Four tagging SNPs and also the 5 SNPs previously described were studied. Genotyping is being performed by SNaPshot, sequencing and RFLP.

We found that women had a statistically significant higher AO than men, either for daughters vs. sons or mothers vs. fathers. Furthermore, mother-son pairs showed a larger anticipation while the father-daughter pairs showed only residual anticipation.

When we focused on genetic modifiers, we found one haplotype in the *TTR* locus, which is more frequent in mutation carriers than in controls individuals. We also found a haplotype that appears to be more frequent in the late-onset patient's group when compared with the early-onset patient's group.

Preliminary results showed that some of the SNPs in *APCS* and *RBP4* genes are significantly associated with AO variation.

These findings confirm anticipation as true biological phenomenon. Furthermore, both parents and offspring's gender were found to be highly significant factors for anticipation. Importantly, in a larger sample of Portuguese families, we expect to disentangle the results found for these genetic modifiers in FAP ATTRV30M AO variability, within and between families which may have an important impact in genetic counselling.

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POSTER 16

Genomic characterization of antimicrobial resistance in *Escherichia coli* isolates from wild European rabbits (*Oryctolagus cuniculus*)

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Antibiotic resistance of bacteria from different habitats has been increasing, mainly as a combined result of the intensive use of antibacterial drugs in human activities and bacterial genetic transfer that allow antibiotic resistant strains to emerge and spread from wildlife sources [1, 2]. Antibacterial agents exert a selection pressure not only on pathogenic bacteria but also on the normal microflora of the intestinal tract of humans and other animals. These populations constitute a reservoir of resistance genes for facultative and obligate pathogenic bacteria [3, 4].

***Escherichia coli* colonize the gastrointestinal tract of many animals and are also commonly found in soil, plants, vegetables and water [5]. These microorganisms are considered important as "indicator bacteria" that can be used to track the evolution of antibiotic resistance in different ecosystems [3].**

The European rabbit (*Oryctolagus cuniculus*) is endemic to the Iberian Peninsula and one of the key species in Iberian ecosystems [6]. Wild rabbit has a considerable economic value as an important game species that is hunted and eaten by humans [7].

The aim of the present study was to analyse the prevalence and genetic basis of antimicrobial resistance in faecal *E. coli* isolates from wild European rabbits in the Azorean Archipelago.

A total of 77 *E. coli* were recovered from 136 faecal samples of wild European rabbits in the São Jorge island at the Archipelago of Azores and analysed for resistance to antimicrobial agents. Different *E. coli* isolates were found to be resistant to ampicillin, tetracycline, streptomycin, sulfamethoxazole/trimethoprim, amikacin, tobramycin and nalidixic acid. The bla_{TEM}, *tet*A, *str*A/*str*B and/or *aad*A, and the *sul*1, *int*I, *int*I2 and *qac*EΔ+*sul*1 genes were demonstrated in ampicillin, tetracycline, streptomycin and sulfamethoxazole/ trimethoprim-resistant isolates, respectively. Similar results were reported in other studies of *E. coli* recovered from wild animals in Portugal [8, 9].

This study showed that *E. coli* from the intestinal tract of wild rabbits can constitute a reservoir of antimicrobial resistant genes that may play a significant role in the spread of antimicrobial resistance.

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POSTER 17

TP53 tumour-suppressor gene analysis in cat mammary gland lesions and metastasis: sequence variants screening and comparative studies

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Feline mammary gland carcinoma has been considered as a suitable model to study human breast carcinoma sharing epidemiological, clinical, morphologic, genetic and prognostic features [1]. In breast cancer, *TP53* mutations are associated with worse overall and disease-free survival, independent of other risk factors, and have been implicated in resistance to anticancer therapies [2]. In cat mammary tumours, this gene has been poorly investigated. Accordingly, our purpose was to isolate and screen the feline *TP53* gene for sequence variations (SVs) detection in cat mammary gland lesions and metastatic samples and to perform comparative studies.

Cat genomic DNA was extracted from 16 non-neoplastic and 39 neoplastic cat mammary gland tissues and from 3 blood and 3 metastatic samples (lung and axillary lymph node). Three *TP53* DNA fragments were amplified using three sets of primers covering exons 2 to 4 (cat*TP53*_2-4 with 572bp), exons 5 to 6 (cat*TP53*_5-6 with 359bp) and exons 7 to 9 (cat*TP53*_7-9 with 657bp). The amplicons were purified, sequenced and analyzed with the Vector NTI software.

In total, we observed 21 SVs. Both in lesions and controls, we found a higher number of transitions. The four SVs identified in the coding region were synonymous. The most polymorphic exon and intron were, respectively, 5 and 7. Three intronic SVs were only observed in carcinomas (introns 2 and 7), one in a fibroadenomatous change (intron 8) and two in control samples (introns 7 and 8).

To our best knowledge, we identified 21 *de novo TP53* SVs in cat mammary masses and this gene revealed to be highly polymorphic, mainly in the noncoding region, both in controls and mammary masses. We did not observe any of the SVs previously reported in cat mammary tumours. None of the major hot spot SNPs/mutations described in breast cancer were identified in our cat mammary samples [3], although a silent mutation was recognized in a hot spot codon (cat codon 155 and human codon 163), suggesting that this DNA region is a genomic fragile site. In fact, when compared with other tumours, in breast cancer we can observe an over-presentation mutation of codon 163 (TAC>TGC) [4] supporting that this investigation gives new insights to the molecular study of the *TP53* gene in cat mammary lesions.

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POSTER 16

Disclosing *Avena* biodiversity through centromeric sequences analysis

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Breeding programs in cereal species has been used to transfer useful agronomic traits to cultivated species. Old varieties and wild species are major reservoirs of these characteristics since human selection decrease varieties genetic diversity.

***Avena* genus is becoming more important for human consumption, livestock feed and as a source of compounds for health and industrial applications [1]. Only four *Avena* species are cultivated, being hexaploid *A. sativa* the specie mostly used for human food and diploid *A. strigosa* for forage. Wild species as *A. sterilis* are used to improve cultivated ones due to their higher growth rates and resistance to rusts, nematodes and herbicides, superior tolerance to abiotic stress situations, higher protein and oil content and better grain yield [2].**

The introduction of these species in selection programs imposes the use of rapid and reliable techniques to identify varieties with interesting characteristics. Molecular markers have been widely used to help in agronomic species characterization and improved traits selection. Several biochemical and molecular markers have been used to clarify phylogenetic relationships in *Avena* genus (reviewed in [3]) and the study of repetitive sequences may be extremely important to unravel *Avena* species genomic diversity.

This work aimed to characterize distinct *Avena* species / varieties namely commercial, traditional and wild lines including diploid A. *strigosa* and hexaploids *A. sativa* and *A. sterilis* using PCR-based technique to target centromeric sequence. Plant centromeres are essential functional chromosome domain particularly rich in repetitive sequences. The results obtained through their molecular study allowed to group all lines characterized accordingly to species and ploidy levels disclosing also a high level of diversity in Portuguese Oat varieties.

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POSTER 11

Whole exome sequencing in patients with a Rett-like phenotype

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Rett syndrome (RTT) is a neurodevelopmental disorder characterized by intellectual disability in association with neurological symptoms, that results in a large fraction of patients from mutations in the *MECP2* gene, usually a point mutation or a small insertion or deletion. However, there is still a large portion of patients that clinically present a Rett syndrome-like phenotype in whom no *MECP2*, *CDKL5* or *FOXG1* mutations are found [1]. Currently, the use of whole exome sequencing (WES) in the clinical setting allows the identification of genes underlying autosomal recessive and autosomal dominant disorders that have until recently lacked gene identification, with a particular contribution for the discovery of new genes associated with intellectual disability [2]. The goal of this project is to identify new genetic causes of Rett-like phenotypes using WES, in a cohort of 20 Portuguese patients (18 female, 2 male) with a clinical presentation significantly overlapping Rett syndrome, according to the recently revised criteria. We used the SOLiD technology for sequencing, after exome capture. In the variant filtering, we excluded variants present in an in-house database of exome sequences (from controls and patients with different disorders, excluding ID), searching for de novo variants in heterozygosity, inherited variants present in heterozygosity in parents but in homozygosity or compound heterozygosity in the patient, and maternally transmitted variants in male patients. We performed bibliography searches using PubMed, and searched for functional and disease related information in the Gene and OMIM databases. We used SIFT, Polyphen, CONDEL, MutationAssessor, MutationTaster and Pmut softwares to predict the impact of the variants found. WES allowed the identification of gene mutations with a very likely contribution for neurodevelopment that may contribute for the pathology in the studied patients. Interestingly, sequence variants in several genes related to ciliopathies, autism and other neurological disorders (including ataxias and neuropathies) were identified. Patients were often carriers of more than one potentially pathogenic mutation, in addition to heterozygous mutations in known recessive disease-causing variants (associated with unrelated phenotypes). With this work we expect to define new genetic – X-linked, autosomal recessive and dominant - disorders resembling Rett syndrome that have until recently been recalcitrant to gene identification.

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POSTER 20

FOXP3 gene: a susceptibility marker in triple negative breast cancer (TNBC)

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A subgroup of malignant tumor that has received much attention is the triple negative breast cancer (TNBC), which presents phenotype of negative estrogen receptor (ER), negative progesterone receptor (PR) and has no overexpression of the oncogene *HER2* (human epidermal growth factor 2). The marker for identify regulatory T cells (Treg) is *FOXP3* gene (forkhead transcription factor 3), a transcription factor that controls the development and function of Tregs, and whose expression may be increased in tumor cells. This study aimed to investigate a genetic polymorphism in *FOXP3* (C/A, rs3761548) gene for a possible involvement in TNBC pathogenesis and prognosis. Genetic polymorphism was evaluated in 50 TNBC patients and in 115 controls individuals free of neoplasia by allele specific PCR (ASO) and analyzed by electrophoresis on acrylamide gel (10%). The association study was performed using contingency tables to calculate the odds ratios (OR) with a confidence interval (CI) of 95%. The genotype frequency for *FOXP3* was 12% (6/50) and 4% (4/115) for AA homozygote, 34% (17/50) and 57% (66/115) for AC heterozygote and 54% (27/50) and 39% (45/115) for CC rare homozygote, in patients and controls, respectively. Patients homozygous AA had a risk almost 4-fold increased for TNBC development, indicating a positive association for these genotype (OR = 3.78; 95% CI =1,02-14,06). When comparing the genotypes of *FOXP3* and the clinical parameters, there was no correlation with the following: tumor size (p = 0.482; rho = 0.102), lymph node involvement (p = 0.890; rho= -0.023) and nuclear grade (p= 0.682; rho= -0.062). In conclusion, *FOXP3* gene, because it was positively associated with TNBC susceptibility appears to be a promising candidate for further investigation in larger samples of this neoplasia and also in other molecular subtypes of breast cancer.

POSTER 21

Cell Biology Services at CEDOC/FCM

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The Cell Biology Services at CEDOC is a non-profitable Cell Biology Platform that offers a range of affordable, custom-made services to the research community. The Platform offers isolation of various types of human blood products (cells and sera). Primary blood cells such as mononuclear cells, monocytes, B cells and T cells are delivered on time either fresh or frozen. Haematopoietic progenitor's differentiation into dendritic cells and macrophages is also available. The Platform is now expanding its services so as to offer genetic manipulation of primary cells.

The products are processed under rigorous sterile conditions and observing strict standard operation procedures resulting from our improved, time-tested team methodologies. During and after isolation procedures, the products are analyzed and quality-tested. This service makes use of assay techniques such as Flow Cytometry, Microscopy, among others. Here, we will give an overview of our standard techniques and present our recent data comparing different methods of cell isolation, and methods for transducing non-dividing primary cells. The impact of those methodologies will be evaluated regarding cell yield, purity, and cell functional characteristics.

A highly qualified and experienced team works, with gold standard criteria, to help researchers of several fields such as Immunology, Oncobiology or Genetics. With this Service we expect to establish collaborations with several Biotech companies and research groups, also to offer unique Cell Biology training to students and to develop in-house technology that may in the future lead to the setting of spin-off companies.

Poster 22

Development of stable lipoplexes DODAC/MO/PEG-FOL for selective delivery, via folate receptor, of therapeutic siRNA

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The evolution of cationic liposomes into one of the most appealing and versatile nonviral vectors for the delivery of nucleic acids [1] and the discovery of RNA interference (RNAi) [2], have made of the development of devices based on cationic liposomes to deliver RNAi molecules to specific cells, an appealing strategy to achieve the treatment of genetic disorders.

Our work consisted in the development and characterization of novel nanometric systems (≈ 100 to 150 nm), for specific delivery of therapeutic siRNA, based on dioctadecyldimethylammonium chloride (DODAC)/ monoolein (MO) liposomes, which previous studies with a similar system developed by our group have validated as a promising vector for plasmid DNA delivery [3]. DODAC/MO vesicles were coated with polyetilenoglycol (PEG) grafted lipids to increase circulating half-life, and PEG-folate grafted lipids to obtain selectivity towards folate receptor expressing (FR+) cells [4], a receptor characteristically overexpressed in several cancer types including chronic myeloid leukemia.

DODAC/MO/PEG liposomes and derived RNA-lipoplexes (liposome-RNA complexes) were produced and shown to be stable in conditions simulating their application by intravenous injection (incubated in solutions of different concentrations of NaCl and H_3O^+ and in fetal bovine serum solutions at 30 e 80% (v/v)). The systems were highly effective in RNA complexation, with efficiencies up to 99%, and the uptake of PEG-Folate coated systems was greater in FR+ cells, comparing to FR- cells and to systems without surface PEG-Folate, indicating selectivity towards the folate receptor.

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POSTER 23

The African genetic input in the Arabian Peninsula: evaluating between pre-historic and historic migrations

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The ''southern coastal route'' model predicts that the early stages of the modern human dispersal out-of-Africa took place when people crossed the Red Sea to southern Arabia. We have recently demonstrated that three minor west-Eurasian mitochondrial DNA (mtDNA) haplogroups (N1, N2, and X), which branch directly from the first non-African founder node, coalesce to the time of the out-of-Africa ~60 Ka, suggesting an ancient ancestry within the Arabian Peninsula. This pattern supports Arabia as the first staging post in the spread of modern humans around the world.

In this work we are focusing on the African lineages which are still observed today in the Arabian Peninsula. Are these lineages descendent from the first migrants of the out-of-Africa, contributing as further evidence for this route of migration, or were they introduced more recently? Previous studies on the mtDNA hypervariable region I described cultural connections in the Red Sea between Arabian Peninsula and East Africa such as human movements resulting from the Arab slave trade from the seventh century onwards. In this work we analyzed the available sub-Saharan complete sequences in African and Arabian/Near Eastern populations, and performed the complete sequencing of 26 samples belonging to the rare haplogroups L4 and L6. The analyses of these complete samples together with 31 samples already published, showed that both haplogroups had an origin in East Africa with an age estimation for the most recent common ancestor of around 89 and 25 Ka for the haplogroups L4 and L6, respectively. When analysing the available complete mtDNA African sequences observed in Arabia and performing founder analysis for the hypervariable region I of these sequences, all sub-Saharan lineages observed in Arabian/Near Eastern populations are derived sequences of recently-evolved sequences in Africa, mostly in East Africa.

This study supports the hypothesis of the slave trade as the main reason for the observation of African lineages in Arabia. The migration out-of-Africa did not leave descents of L haplogroups in Arabia – only the derived N and M lineages are observed.

POSTER 24

The angiogenic factor VEGF-A in canine cutaneous melanocytic lesions: evidence of distinct expression patterns according to tumor diagnosis

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Melanocytic tumors (MTs) occur as much in humans as in dogs and their incidence has been increasing. Cutaneous MTs are mostly benign but, when malignant, they present a high mortality rate and are frequently associated to angiogenic events, which facilitate tumor growth and metastasis. The vascular endothelial growth factor – A (VEGF-A) is a protein produced by endothelial and inflammatory cells that binds to a VEGF receptor (VEGFR) of the vascular endothelium. Normally, it leads to angiogenesis, through cell proliferation, migration and survival. However, the upregulation, gene amplification and/or mutations of VEGF-A have been observed in several canine and human cancers, due to the overstimulation of angiogenesis, being associated with poor prognosis and representing a good therapeutic target. Overexpression of VEGF-A by tumor and stromal cells has been observed in both primary and metastatic human malignant melanoma (MM); however, studies concerning its role in canine MTs, particularly in cutaneous subtype, are limited.

Therefore, and since the dog has been considered a good model for studying MM, in this study, we aimed to assess VEGF-A expression in 45 canine cutaneous MTs and its association with tumor diagnosis, using immunohistochemical techniques. The labeling extension (% of positive labeled cells), intensity and location were recorded, and the statistical Chi-square test was used.

The VEGF-A was detected in blood vessels and tumor and inflammatory cells. Regarding labeling extension, both melanocytomas and MMs largely presented a diffuse staining pattern ($p=0.162$). On the other hand, the differences for both labeling intensity and location were statistically significant ($p<0.001$): in melanocytomas the intensity was mainly weak, with a simultaneous cytoplasmic and nuclear staining (11 of 15 positively labeled melanocytomas, for each criterion); and in MMs it was mainly moderate and strong (54% and 36% of cases, respectively), with a cytoplasmic labeling pattern (93% of cases).

Our results show that VEGF-A is expressed in the generality of MTs, which is in accordance with the fact that both benign and malignant lesions can be dependent on angiogenesis to grow and survive. Its detection in tumor cells can also indicate that VEGF-A may induce their proliferation through autocrine and/or paracrine loops, in addition to its angiogenic functions. Moreover, the differences observed in the labeling intensity suggest its association with tumor aggressiveness, since it is more intensely expressed in MMs. Thus, this study supports the importance of VEGF-A as a mitogenic/angiogenic marker in canine MM, indicating that it might be useful for diagnostic purposes and a good therapeutic target in this type of cancer. The biological significance of the nuclear staining pattern observed in melanocytomas is unclear. Furthermore, our results are similar to analogous studies in human counterpart, reinforcing the potential of dog as a model for studying MTs.

POSTER 25

Is VEGFR-2 related to canine melanocytic tumors' behavior? A preliminary study in cutaneous lesions

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Melanocytic tumors (MTs) are relatively frequent in humans and dogs and their incidence is increasing. Though cutaneous MTs are usually benign, malignant melanomas (MMs) are very aggressive and frequently metastatic. Angiogenesis is related to tumor malignancy, since it enables its growth and metastasis. In normal tissues, the vascular endothelial growth factor receptor - 2 (VEGFR-2), a receptor tyrosine kinase (RTK) of the vascular endothelium, can induce angiogenesis by stimulating the proliferation, migration and survival of these cells, after its activation by VEGFs. On the other hand, its upregulation, gene amplification and/or mutations have been related to many human and canine cancers, due to the excessive stimulation of angiogenesis, being associated with poor prognosis. Also, this receptor has been a target in clinical trials with RTKs inhibitors. In human primary and metastatic MMs, this receptor is often overexpressed by tumor and endothelial cells, but similar studies regarding its role in canine MTs are scarce.

On that basis, and since the dog has been considered a good model for studying MM, we aimed to evaluate immunohistochemically the VEGFR-2 expression in 45 canine cutaneous MTs and its association with tumor diagnosis. The labeling extension (% of positive labeled cells), intensity and location were recorded, and the Chi-square test was used. As far as we know, this is the first immunohistochemical study of VEGFR-2 in canine MTs.

The VEGFR-2 was detected in tumor and endothelial cells. Regarding labeling extension, both melanocytomas and MMs generally presented a diffuse expression ($p=0.227$). Contrarily, labeling intensity showed statistically significant differences ($p<0.001$): in melanocytomas it was mainly weak, while in MMs it was mostly strong (77% and 64% of cases, respectively). With respect to VEGFR-2 location, melanocytomas mainly presented a simultaneous nuclear and cytoplasmic staining, whereas MMs mostly showed a cytoplasmic labeling pattern; despite this tendency, it was not statistically significant ($p=0.065$).

The expression of VEGFR-2 in the blood vessels of benign and malignant MTs supports the fact that, in both cases, tumor cells can depend on angiogenic events to grow and survive. Furthermore, its detection in tumor cells, which normally do not express it, indicates that it may also induce their proliferation, through autocrine and/or paracrine VEGF signals. Moreover, the differences observed in the labeling intensity suggest that VEGFR-2 expression may be related to tumor malignancy, since it is strongly expressed only in malignant lesions and in the majority of these cases. Therefore, VEGFR-2 may be a criterion of clinical aggressiveness and a useful diagnostic marker, as well as a potential target for anti-RTK therapy in canine MM. Finally, our data are comparable to the observations made in human studies, highlighting the value of dog in comparative oncology, particularly in MTs.

IRAP diversity in *Fagaceae* species - perspectives for LTR universal primers

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Retrotransposons (RTNs) are common on plant genomes, showing activity at transcription and integration levels. RTNs have been used as markers because their integration creates new joints between genomic DNA and their conserved ends. These insertion polymorphisms, their abundance, dispersion and faculty to transpose make them useful molecular markers to access genetic diversity in plants.

Inter-retrotransposon amplified polymorphisms (IRAPs) are multilocus markers that amplify the genomic DNA between two long terminal repeat (LTR) retrotransposons revealing polymorphisms. IRAPs have been widely used for evolution, genetic diversity assessment and DNA fingerprinting studies.

Molecular studies on *Fagaceae* are focused on a reduced number of species with economic importance. Thus, it is important to extend the molecular knowledge to other species to better understand their phylogenetic relationships.

In this study, we tried the amplification of 6 universal LTR primers [1] in 27 individuals from *Quercus*, *Castanea* and *Fagus* genera, in order to evaluate their genetic variability and phylogenies with IRAPs. Out of the 21 possible primer combinations involving the 6 LTR universal primers, we selected 5 based on the number of amplified bands and/or IRAP polymorphism. The 5 primer combinations were used in 2 species from *Castanea*, 3 species from *Fagus* and 22 species from *Quercus* belonging to the taxonomic sections *Quercus* (6), *Cerris* (5), *Lobatae* (8) and *Mesobalanus* (2), in a total of 27 individuals.

The IRAP markers produced a total of 283 bands and 100% of polymorphism. On average each primer combination produced 57 bands. The IRAP bands ranged from 130 to 3670 bp. Six monomorphic bands were amplified only in the oak section *Quercus*, and will be further studied in detail once they could constitute specific markers for this *taxon*.

Based on the pool of the IRAP data, an UPGMA dendrogram was constructed. The genetic similarity among the 27 individuals ranged from 66.5% to 88.5%. These markers did not cluster the individuals per *genus*. However, most of the oaks were grouped by taxonomic section. Chestnuts and beechs were properly clustered with 86.5% and 80.8% of similarity, respectively. Oaks from the *Cerris* and *Quercus* sections showed a similarity of 75.2% and 67.8%, respectively. These sections were clustered separately but an intromission of oaks from the section *Lobatae* was observed in both sections. *Quercus phillyraeoides* has not been yet classified in one of the existent taxonomic sections. This fact was confirmed by IRAP technique performed here that also clustered it as branch.

IRAP proved to be suitable for DNA fingerprinting, estimation of phylogenies and were specific enough in the clustering of the *Fagaceae* individuals according to the taxonomic criteria.

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POSTER 27

Lymphocytes and dendritic cells in Mucopolysaccharidosis type VI patients

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Lysosomal storage diseases (LSD) are a group of rare hereditary metabolic disorders caused by accumulation of undegraded molecules in the lysosome, mainly due to the impairment of the function of lysosomal enzymes. Mucopolysaccharidoses are LSDs characterized by the accumulation of glycosaminoglycans. The focus of this work is Mucopolysaccharidosis type VI (MPS VI), which is a disorder caused by mutations in the gene encoding the ASB enzyme (*ARSB*). In this disease there is accumulation of the glycosaminoglycan dermatan sulfate. The lysosome is an important organelle in the presentation of lipid antigen to T cells. Invariant natural killer T cells (iNKT) are the most studied lipid specific T cells and are well known by their immunoregulatory properties.

In this work seven MPS VI patients from Metabolic Diseases Unit, São João Hospital and seven control subjects from Blood bank, São João Hospital were analyzed. All patients analyzed were under Enzyme Replacement Therapy. For this study B, T and iNKT peripheral blood lymphocytes were analyzed by flow cytometry. For three of the patients dendritic cells (DCs) were differentiated from monocytes and their phenotype was analyzed by flow cytometry. For lipid antigen presentation assays three patients were analyzed.

In MPS VI patients we found no alterations in the percentage of the T and iNKT cells and in their subsets according to CD4 and CD8 expression. These parameters have been previously analysed for other LSDs in our laboratory and imbalances in the iNKT cell subsets were found (Cátia Pereira et al. 2013, Molecular Genetics and Metabolism and unpublished results). The results obtained with the MPS VI patients highlight the fact that lysosome dysfunction *per se* is not sufficient to induce iNKT subset alterations. For B lymphocytes we found an increase in the percentage of these cells in MPS VI patients when compared with control subjects. For dendritic cells phenotype three patients were analyzed. For two of them we found a decrease in the expression of CD1a, CD11c and HLA-DR (MHC-class II). In lipid antigen presentation assays, three patients were tested for the capacity of their dendritic cells to present lipid antigens by the CD1b molecule. We found no alterations in patients' capacity to present the lipid antigen GM1 by the CD1b molecule.

At the moment we are actively recruiting more patients and also collecting clinical data in order to perform association studies.

Application of the PowerPlex® Y23 System for Forensic Purposes in the Portuguese Population

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The 23 Y-chromosomal short tandem repeats (STRs) included in the PowerPlex® Y23 System (Promega) (DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456, GATA H4) were typed in 250 samples from Portugal. A total of 236 different haplotypes were found, among which 14 haplotypes were shared by two individuals. The overall haplotype diversity (HD) was 0.9996. Since this sample had been previously typed with the AmpFISTR YFiler Amplification Kit (Applied Biosystems), a comparison was undertaken in terms of concordance and haplotype diversity (HD). Two discrepancies were found between both kits for the loci DYS385 (silent allele) and GATA H4 (sequence variant). Assessment of the influence of each locus in HD increment in this sample was also undertaken: by fixing the haplotypes generated with the YFiler loci and adding the six new PowerPlex Y23 loci one by one, the markers that contribute more are DYS576 and DYS481. In this sample, the same HD was obtained with or without the DYS549 and DYS643 loci. Population comparisons with available North-American data were undertaken, namely with the Caucasian, Hispanic, African and Asian subpopulations. After Bonferroni correction for multiple comparisons, significant differences were found between all pair of subpopulations except between our sample and the Caucasian American one.

Genes under estrogenic control in the testis: impact on apoptosis and male fertility

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Estrogens are classically female hormones, however over the last years experimental and clinical evidences have highlighted for their role in testicular function. As liposoluble steroid hormones, estrogens exert their effects by interaction with intracellular receptor proteins, which act as transcription factors controlling the expression of target genes. In the testis, apoptosis is a key event strictly maintaining the appropriate ratio between germ cells and Sertoli cells, the somatic cells that support germ cell development and spermatogenesis. It has also been suggested that Sertoli cells play a crucial role controlling germ cells fate, by secretion of survival and death factors, which act on receptors in germ cells, or establishing direct cell-to-cell membrane contacts. These include the survival factor desert hedgehog (Dhh), the stem cell factor (SCF) and its receptor the c-kit, as well as the death factors Fas-Ligand (Fas-L) and Fas-receptor (Fas-R). Also, apoptosis regulators Aven and regucalcin (RGN) have been shown to be expressed in Sertoli and germ cells. Although these factors have been described as estrogen-target genes in a variety of tissues, the effect of estrogens controlling their expression in the testis has remained unknown. We hypothesize that estrogens may influence germ cell survival or death indirectly by governing the expression of the aforementioned factors. In the present study rat testis were cultured *ex vivo* in presence or absence of 100nM of 17β-estradiol (E_2), and the expression of Dhh, SCF, c-kit, Fas-L, Fas-R, Aven and RGN was studied by means of real-time PCR and Western blot analysis. E_2 down-regulated the c-kit expression while increasing expression of its ligand, SCF. Also the expression of anti-apoptotic proteins, Aven and RGN, was increased in response to E_2. Our results demonstrated that the estrogenic control may modulate Sertoli:germ cell communication affecting germ cell survival and apoptosis, which could have a pronounced impact on male fertility.

POSTER 30

The evolution of cold tolerance in *Drosophila*: from phylogenetics to gene expression

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Temperature is one of the major environmental factors affecting geographical distribution of ectotherm species, such as, Drosophilids. Therefore, the different capacity of species to tolerate extreme temperatures will influence their distribution across distinct climatic regions. The ability to recover after short term exposure to chill coma inducing temperatures (chill coma recovery) has been widely used as a proxy to determine the cold tolerance of *Drosophila* species. It has been shown that temperate species recover faster than tropical ones but the molecular basis responsible for these differences remains largely unknown. The most credible candidate gene involved in short term cold tolerance is *Frost*. This gene was shown to significantly affect recovery from chill coma in *D. melanogaster* and it reaches its maximum expression levels after two hours of recovery from cold shock. *Frost* up-regulation is significantly lower in *D. americana* than in *D. melanogaster* revealing major differences regarding the molecular basis of cold tolerance in distantly related *Drosophila* species. So, in this study our main goal is to address the evolution of climate adaptation in *Drosophila* and explore the putative role of *Frost* on the basis of adaptation to different climate conditions.

For this purpose we have first estimated separately the phylogenies of 218 and 122 species of the *Drosophila* and *Sophophora* subgenera, respectively. Ancestral reconstruction of climatic distribution indicates that the ancestral species of these two subgenera had a tropical distribution and adaptation to temperate climates has occurred several times independently. We have then performed chill coma recovery experiments and gene expression assays of *Frost* for six species, representative of the major groups of *Drosophila*, from different climatic distributions, with fully sequenced and annotated genomes. For tropical and cosmopolitan species with tropical origin, chill coma recovery times increase linearly with cold exposure times, whereas for temperate and cosmopolitan species with temperate origin, recovery times are independent of exposure times. Lower fold changes in *Frost* expression after two hours of recovery after cold shock are always associated with temperate species. The ability to largely increase *Frost* levels after cold shock should be a feature of the tropical ancestral of *Drosophila* genus and these levels should be reduced after the transitions to temperate climates. However, we suggest that this reduction is higher for species belonging to lineages that are temperate for longer periods of time.

POSTER 31

Genotoxicity of lead in *Lactuca sativa* L. root cells measured by the comet assay

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During the past few decades, heavy metals, an important group of environmental pollutants, have received increasing attention. As these metals are non degradable, they can easily enter in the food chain and then endanger human and animal health. Lead (Pb) is one of the most abundant heavy metals being highly phytotoxic, often causing an accentuated decline in crop yields.

***Lactuca sativa* L., a very important crop used in human diet, is recommended as a standard species for tests of toxicity and genotoxicity. In this study, plants of "Reine de Mai" cultivar were exposed to different concentrations of Pb (0, 0.05, 0.5, 10 and 20 ppm), and grown hydroponically under controlled conditions for 28 days. Lead genotoxicity was analysed in root apical cells using an alkaline comet assay - a sensitive method for detection of DNA damage - described by Gichner *et al.* [1] with slight modifications. Slides were stained with ethidium bromide and examined with a Nikon Eclipse 80i microscope, with an excitation filter of 510-560 nm and a barrier filter of 590 nm. The DNA damage was quantified by the percentage of DNA in tail.**

The results clearly indicate that lead induced DNA fragmentation in a dose-dependent manner, with a maximum effect at 10 ppm (52.63% ± 2.50). However, there was a decrease in the % of DNA in tail at 20 ppm concentration. The concentration of 0.05 ppm was not genotoxic to this cultivar (7.72% ± 2.56).

This assay reinforces *Lactuca sativa* L. as an appropriate crop to use in genotoxicity and toxicity tests, due to its concentration-dependent response to the different Pb concentrations tested.

The comet assay proven to be an effective and suitable method to detect lead-induced DNA damage, through the assessment of comets presence. Therefore, this technique can be used in toxicity studies, in different plants, to evaluate DNA damages induced by several agents, like heavy metals.

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POSTER 32

An mtDNA chronology for the Out-of-Africa migration

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In the last decades evidence for an origin of modern humans in Africa has been accumulating from different scientific areas like Genetics, Palaeontology and Archaeology. However it is still a matter of debate the timing of the Out-of-Africa migration and to know if it was a single or multiple events. One prominent question is to know if ascendants from present day modern humans have exited Africa before the catastrophic Mount Toba explosion around 74 thousand years (ka) ago.

Using the maternally inherited mitochondrial DNA (mtDNA) we were able to establish a reliable chronology for the migration out of Africa using a molecular clock that takes into account the affect of purifying selection [1]. All modern humans outside Africa descend from two branches of the human mtDNA tree named haplogroups M and N. These two lineages are part of a clade of African origin labelled haplogroup L3 that represents the most ancient link between Africans and Non-Africans and dates between 65 to 70 ka [2] suggesting a migration after this time. The Out of Africa haplogroups M and N are equally found from Arabia/India to Australia suggesting a simultaneous expansion of both clades through a Southern Costal route [3]. European mtDNA gene pool indicates that Europe's first settlement represents a later Northern offshoot of this migration since all the lineages are derived from Arabian/Near Eastern variation [4]. While the age of L3 at 65-70 ka represents the upper bound of the estimate of migration, the Outside Africa haplogroups M and N date between 60 to 50 ka in Arabia and India [5,6] the first stepping points of modern human expansions across the whole globe. The expansion times of M and N at this time also correlates well with the first archaeological signals that could be assigned securely to modern humans [6]. That means that previous signals in the archaeological record that have been pointed out to be the product of modern humans between 130 to 75 ka were probably produced by archaic species or at most by groups of modern humans that did not survive till the present.

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POSTER 33

Molecular Genetics in Cyanotoxin Research – An Overview

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Cyanobacteria are photosynthetic organisms that exist on Earth for over 3 billion years. Inhabiting a wide range of ecological niches its major relevance includes the production of dense blooms under eutrophic conditions and the synthesis of secondary metabolites designated cyanotoxins, including hepatotoxins (microcystin and cylindrospermopsin), neurotoxins (saxitoxin and anatoxin) and dermatoxins. Cyanobacteria existence is commonly associated with cases of intoxications and death in both humans and animals that contact with these toxins and are also capable of degrading water quality. Because of their toxicity and mode of action in the environment and organisms, it is important to have an efficient and rapid monitorization process to rapidly detect cyanotoxins potentially-producing species. Until recently, cyanotoxins detection was accomplished recurring to the use of microscopy and chemical methods. However, the identical morphology of cyanotoxins producing and non-producing cyanobacteria challenged the toxicity studies, creating the need to development molecular techniques capable to quantify and qualify those toxic compounds and their producing species. Techniques such as the Polimerase Chain Reaction (PCR), which is based in the DNA amplification that occurs *in vitro*, allowed the detection of cyanotoxin-producing microorganisms in a rapid, precise and technically accessible way, due to the use of colonies, filaments, whole cells and environmental samples. Therefore the main objective of this study is to provide an overview of the several molecular methods that have been used in cyanotoxin research focusing mainly in the PCR reaction and the already available techniques developed for each cyanotoxin. Here we will discuss the relevance of these methods in comparison with conventional methodologies and conclude that now is possible to study and monitor the presence of cyanotoxins in several types of matrices and differentiate the cyanotoxins producing from non-producing species, in a more rapid and specific way than when using conventional methods.

Keywords: Cyanobacteria, Cyanotoxins, Molecular Methods

POSTER 34

Evaluation of antimicrobial activity of Portuguese spices and essential oils against pathogenic and spoilage bacteria

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The evaluation of antimicrobial activity of essential oils extracted from a variety of aromatic plants, and several spices often used in the Portuguese gastronomy was studied *in vitro* by the agar diffusion method.

The essential oils of bay, cinnamon, coriander, cumin, lemon, garlic, ginger, marjoram, nutmeg and parsley, and several spices (lime, lemon, commercial lemon juice, hot chili, vinegar, olive oil, extra virgin olive with garlic and olive oil balsamic) were tested against Gram-positive (*Listeria monocytogenes*, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*) and Gram-negative strains (*Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*).

For most essential oils and spices examined, *B. cereus* was the most susceptible bacteria, while *S. epidermidis* showed, in general, least susceptibility. Most of the essential oils and spices considered in this study exhibited a significant inhibitory effect. Among the ten essential oils evaluated, cinnamon showed the greatest antimicrobial activity. With respect to the Portuguese gastronomy spices, the white lemon showed the highest antimicrobial activity.

Cinnamon oil showed a promising inhibitory activity even at very lower concentrations, thus revealing its potential as a natural preservative in food products against several causal agents of foodborne diseases and food spoilage. In general, the results demonstrate that, besides flavoring the food, the use of aromatic herbs and other flavorings in gastronomy can also contribute to a bacteriostatic effect against pathogens.

Duplex Polymerase Chain Reaction Assay for Early Detection of *Mycobacterium tuberculosis* Complex in Tuberculosis Diagnosis

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Tuberculosis (TB) has become more prevalent in recent years. The *Mycobacterium tuberculosis* (MTB) complex is responsible for 8 million new TB cases and more than 2 million deaths every year [1]. In Portugal in 2011, 2388 cases of TB were diagnosed, which unlike all other western European countries is above the low incidence rate. Nontuberculous mycobacteria (NTM), a major cause of opportunistic infections in immunocompromised individuals [2], demand special attention because most of them are inherently resistant to currently available drugs [3]. Early detection and identification of the type of mycobacteria causing infection are required to optimise therapy and manage patients appropriately. TB diagnosis relies on the "gold standard" culture procedure with direct observation and comparison to conventional stains, followed by solid culture to test for antimicrobial susceptibility. These methods, which require sufficient bacterial growth, are not routine in every laboratory so can be inaccurate, non-reproducible, and above all, time-consuming [4]. In order to complement these classic yet standard procedures, new, reliable and prompt molecular alternatives are needed for effective disease diagnosis [5].

Here we used two distinct and highly conserved genetic markers: *hsp*65, a transversal marker for the *Mycobacterium* genus [6], and *cfp*32, described as being specific for the MTB complex [7]. Genetic material from bacteria was obtained directly from clinical samples from different hospital facilities from Northern Portugal. PCR amplification of these markers was simultaneously performed on 127 isolates. Complex and non-complex isolates were clearly distinguished. A total of 117 isolates were identified as MTB complex and 10 isolates as NTM. All amplification products from both markers were confirmed by sequencing. No polymorphisms were found among all *cfp*32 products, confirming the specificity and exclusivity of this marker to MTB complex.

Using reference strains and known negative controls, we developed a specific duplex-PCR for identification of the genus and complex of isolates directly obtained from biological samples without the need for culture. The method for accurate TB diagnosis presented here can be performed in less than one day, unlike classic methods used routinely in clinical laboratories.

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POSTER 36

Autophagy induction as a therapeutic target for Machado-Joseph disease: 17-DMAG and lithium chloride

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Machado-Joseph disease (MJD) is an inherited neurodegenerative disease, caused by a CAG repeat expansion within the coding region of *ATXN3* gene, resulting in a mutant protein - ataxin-3 – bearing an expanded polyglutamine tract that forms intranuclear inclusions and has neurotoxic properties. The accumulation of misfolded proteins in neurons, leading to the formation of cytoplasmic and nuclear aggregates is a common theme in age-related neurodegenerative diseases, possibly due to disturbances of the proteostasis and a decreased activity in cellular clearance pathways. Treatment of neurodegenerative diseases with drugs that up-regulate autophagy, a lysosome-dependent cellular mechanism responsible for the turnover of intracellular organelles and long-lived proteins, has shown promising results. We developed a mouse model of MJD (CMVMJD135) which has a robust behavioral phenotype and specific neuropathology, providing a framework for pre-clinical trials. 17-DMAG, a brain-permeable HSP90 inhibitor and autophagy inducer, was administered chronically to CMVMJD135 mice and markedly delayed disease progression, decreased ATXN3 aggregation and the levels of the mutant protein, possibly through autophagy induction. Lithium is a well-known autophagy inducer, exerts neuroprotective effects in different conditions, and has been proposed as a promising therapeutic agent for several neurodegenerative diseases. Therefore, we also tested the efficacy of chronic lithium treatment in the CMVMJD135 mice. Surprisingly, lithium treatment, in spite of activating autophagy, showed limited effects in the rescue of the motor deficits observed in this mouse model. In summary, both 17-DMAG and lithium chloride were able to induce autophagy in the brain, but only 17-DMAG had a strong effect in the improvement of CMVMJD135 mice symptoms, suggesting it a promising compound for the treatment of MJD, while lithium had limited effects. Identification of the differences in the mechanisms of action of these two compounds should enlighten therapy development for MJD.

POSTER 37

Discrimination of grapevine varieties using Short Tandem Repeats

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The grapevine (*Vitis vinifera* L.) is a diploid plant that has a relatively small genome size (475-500 Mb) organized in 19 chromosomes [1]. This plant is one of the most valuable and oldest horticultural crops used to produce table fruit, raisins, juice and wine [2-4]. The nomenclature of grape varieties has resulted in a complex pattern of synonyms (different names for the same variety) and homonyms (different varieties identified under the same name) [2].

Variety identification is important to grape growers, winemakers, regulatory authorities, and consumers [5]. The increasing interest in cultivar identification results from the need of planting and producing grapes and derived products that are correctly identified, due to the economic value of conformity with protected designations of origin [5-7]. Accurate identification of grapevine cultivars is crucial and so is the importance of a good traceability system throughout the winemaking process, including variety provenance [5].

Our goal is to establish a reliable genetic identification method that can be applied throughout all stages of the wine production process (including the nursery, the vineyard, and winery stages), based on autosomal short tandem repeats. In order to accomplish that, we propose to review and fully characterize a set of 8 STR markers distributed through 5 chromosomes recommended by the International Organization for Vine and Wine (http://www.oiv.int/oiv/cms/index?lang=en) and utilized in The European *Vitis* Database (http://www.eu-vitis.de/index.php), by sequencing alleles in 12 Portuguese varieties.

Additionally, we propose the use and characterization of 4 newly described STR markers retrieved from the *Vitis vinifera* whole genome sequence, available at http://www.plantgdb.org/VvGDB/.

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POSTER 38

NAPRT1 and NAMPT expression and regulation in human tumors

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Nicotinamide phosphoribosyltransferase (NAMPT) and nicotinate phosphoribosyltransferase 1 (NAPRT1) play a key role in the cellular metabolism, as main nicotinamide adenine dinucleotide (NAD) salvage enzymes in humans. More than a cofactor in redox reactions, NAD is a substrate for NAD-consuming enzymes, namely PARPs and sirtuins, that are involved in mechanisms such as gene silencing, DNA repair and cell signaling [1]. Since NAD levels are essential for cell survival and cancer cells have increased NAD requirements [2], both NAMPT and NAPRT1 are targets for the development of novel anti-cancer therapies [3, 4]. In this study, we present the results of NAPRT1 and NAMPT expression in several different human tissues and tumor cell lines and show the diversity of expression patterns between tissues at both mRNA and protein levels. Also, we characterize novel NAPRT1 transcripts and discuss some of the mechanisms involved in the regulation of expression of the NAPRT1 gene.

In conclusion, our findings highlight that expression profiles of both NAMPT and NAPRT1 should be taken in consideration for the use of nicotinic acid as cytoprotective in therapies using Nampt inhibitors.

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Bacterial characterization by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and bioinformatic tools

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***Enterococcus* spp. and *Escherichia coli* are commensal microorganisms from the gastrointestinal tract of humans and animals that are sometimes also considered as major pathogens. The excessive use of antibiotics may be an important factor in the natural selection of bacterial resistance in such pathogenic and commensal bacterial populations [1].**

Bacteria can be rapidly identified using the polymerase chain reaction (PCR), but even this sensitive technique does not provide sufficient information to unambiguously discriminate between species that requires amplification of specific genes [2]. The number of publications in the NCBI Pubmed database describing the use of matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) to identify and characterize microbes has doubled since 2007. By early 2012, 40 articles described successful applications of mass spectrometry in this field [3]. The MALDI-TOF-MS technique is expedient, precise and cheaper than conventional microbiological and/or molecular biology methods. MALDI protein fingerprinting may also be a rapid way of characterizing antibiotic resistance in pathogenic bacteria. The presence of *E. coli* β-lactamase can be readily deduced by detecting the products resulting from lactam ring hydrolysis of antibiotics by MALDI-TOF-MS. This type of analysis is very fast as results can be obtained within 4 hours [4].

In this study, 120 enterococci and 120 *Escherichia coli* isolates from wild birds of the Archipelago Azores were analysed through a streamlined protocol going from the seeding of bacteria up to the bioinformatics study of the MALDI-TOF-MS data obtained. Preliminary results are promising. Using the bioinformatics tool mMass it was possible to compare 1200 mass spectra. From 600 spectra from *Enterococcus* spp. isolates, it is possible to distinguish four main groups corresponding to the four enterococci subspecies *E. faecalis*, *E. faecium*, *E. hirae* and *E. durans*. The other 600 spectra from *E. coli* isolates are characteristic of the species. It is feasible that with other bioinformatics tools these spectra will discriminate between peaks associated with antibiotic resistance present in the isolates. Furthermore, studies have shown that despite the high number of spectra obtained by this method, it is possible to detect common peaks among mass-related isolates, suggesting the possibility of identifying specific biomarkers of bacterial characteristics.

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POSTER 40

Analyses of SSR hybridization patterns in *Hordeum* sp. chromosomes using ND-FISH

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Non-Denaturing Fluorescence *In situ* Hybridization (ND-FISH) technique has been used in chromosome spreads of plant species such as barley, in order to study the organization and evolution of microsatellite sequences (SSRs). This methodology is fast when compared with conventional FISH and presents comparable hybridization results.

In this study, we aimed to analyze and compare the hybridization patterns of 16 SSR probes in mitotic metaphase chromosome spreads from *Hordeum vulgare* L., (cultivated barley; H^vH^v; 2n=2x=14), and in the amphiploid tritordeum (X *Tritordeum* Ascherson et Graebner; $AABBH^{ch}H^{ch}$; 2n=6x=42) which resulted from the cross between *Hordeum chilense* L. and durum wheat (AABB; 2n=4x=28), using ND-FISH technique.

Out of the 16 SSR probes tested, only five [$(CAC)_5$, $(AAC)_5$, $(CT)_{10}$, $(AGA)_5$, $(AG)_{10}$], showed hybridization in *H. vulgare*, mainly at the centromeric region. Among these probes, only the $(AAC)_5$ and $(AG)_{10}$ hybridized strongly in the *H. chilense* chromosomes of tritordeum. The probe $(AAC)_5$ presented strong hybridization in the centromere of all *H. vulgare* chromosomes, while in tritordeum it showed weak and dispersed signals in wheat and H^{ch} chromosomes. The sequence $(AG)_{10}$ presented a hybridization pattern similar to that of $(AAC)_5$ in *H. vulgare*. In tritordeum, this probe showed a centromeric location in most of the tritordeum chromosomes, and showed a telomeric location in one pair of wheat chromosomes (genomes A or B). Once the probe $(AG)_{10}$ hybridized more strongly in tritordeum chromosomes, it was performed a reprobe of the same spreads with a genomic probe of *H. chilense* (GISH) for parental genomes discrimination, and to achieve an accurate evaluation of the hybridization pattern on the H^{ch} chromosomes. After reprobing of tritordeum chromosome spreads with the 45S ribosomal DNA (rDNA) sequence - pTa71, by ND-FISH, it were detected all rDNA *loci* being useful for further construction of an $(AG)_{10}$ karyotype. In general, it were detected differences on the hybridization patterns of the probes $(AAC)_5$ and $(AG)_{10}$ among the cultivated and wild barley chromosomes. These differences could be due to evolutionary events that occurred since the common ancestor and which contributed for their speciation or it could occurred more recently in the H^{ch} genome during the formation of the amphiploid tritordeum.

This study revealed the advantages and utility of ND-FISH technique in the study of organization, distribution, and evolution of SSR sequences on amphiploids and respective parents.

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Development of molecular tools for yield associated genes in Portuguese grapevine varieties

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Grapevine is the most important agro-economical fruit crop in Portugal, where over 300 varieties are used for wine production and large experimental populations of clones from various traditional Portuguese varieties are maintained and studied. From a research perspective, these grapevine varieties and clones provide an immeasurable pool of genetic variation for functional genomic analysis and development of molecular tools for viticulture. Previous transcriptomic analysis with Affymetrix GeneChip® *Vitis vinifera* Genome Arrays and Quantitative Real Time Reverse PCR identified five genes with yield associated differential expression profiles in Touriga Nacional, Negra Mole and Arinto clones. Of these genes, four were down-regulated and one was up-regulated in high producing clones in at least two of the three varieties. This study intends to identify Single Nucleotide Polymorphisms (SNPs) and small insertions/deletions (indels) between high and low producing clones. For this purpose, three genes identified as being down-regulated in high producers were chosen for in depth sequence analysis of upstream enhancer and promoter sequences (5´ UTR), as well as intronic sequences. Genomic DNA from six high producing and five low producing Touriga Nacional clones were utilized for PCR with a total of five primer sets for upstream UTR sequences and ten primer sets for intronic sequences. PCR products were sequenced, indicating high levels of heterozygocity between alleles. We were therefore not capable of identifying SNPs or indels between high and low producers in these genes. For further analysis of the molecular processes involved in yield and the development of a screening method for productivity in grapes, the GeneChip® miRNA 3.0 Array was recently used with total RNA from high and low producing clones from the three varieties. Results identified three main miRNAs that may play an important role in *Vitis Vinifera* productivity. We are currently analysing the corresponding target genes of these miRNAs to design gene specific primers and determine yield associated differences in expression by Quantitative Real Time Reverse PCR.

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Chloroplast DNA haplotypes in Iberian *Silene scabriflora* subspecies

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Iberian Peninsula is considered as one of the most important Pleistocene glacial refugia in the European subcontinent and its physiographic complexity and geographical position favored species survival throughout this glacial period.

***Silene* L. genus has about 700 species divided into 44 sections, mostly herbaceous, with world-wide distribution, mainly in the northern hemisphere. Two major centers of diversity are known: one in the Eastern Mediterranean and another in Southwest Asia Regions.**

The present study focuses on *Silene* sect. *Scorpioideae* species. To date, this is the first study concerning genetic diversity and differentiation of *S. scabriflora* subspecies using chloroplast DNA markers. Chloroplast genome (cpDNA) analysis is considered a powerful tool for phylogenetic and phylogeographic studies.

Five pairs of cpSSR (Chloroplast Simple Sequence Repeat) primers were used to amplify 24 accessions of four Iberian *Silene scabriflora* subspecies, namely eight accessions of *S. scabriflora* subsp. *megacalycina* (from Lugo and Ourense, Spain), six of *S. scabriflora* subsp. *scabriflora* (from Castelo Branco, Portugal and Malaga, Spain), six *S. scabriflora* subsp. *tuberculata* (from Malaga, Spain) and four accessions of *S. scabriflora* subsp. *gallaecica* (Coruña, Spain). The distribution of target subspecies extends from North to South of Spain and Western Center of Portugal, covering almost the entire area of the Iberian Peninsula.

Three of the five cpSSR loci were polymorphic. The two different alleles found in each polymorphic locus were combined in two different haplotypes. The most frequent haplotype (87.5%) was found in all the subspecies. *Silene scabriflora* subsp. *scabriflora* samples revealed different haplotypes according to geographic origin: samples from Castelo Branco had the more frequent haplotype and those from Malaga revealed a specific haplotype. Having chloroplast genome a conserved nature, it is an important result the discovery of two different chloroplast haplotypes within the same subspecies. In this way, further chloroplast molecular markers analysis as well as morphological characterization will be undertaken.

Molecular evaluation of aluminum-induced stress on *Plantago almogravensis*

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Aluminum (Al) is the third most abundant metallic element in the earth's crust and is a component of many soil minerals. When soils acidify (pH below 5.5), either naturally or as a consequence of industrial and agricultural processes, Al is solubilized into the toxic Al^{3+} form. Al bioavailability and consequent toxicity is one of the major limiting factors for plants development. However, the genetic basis and molecular mechanisms underlying the physiology of Al tolerance are unclear.

Plantago almogravensis **Franco is an endangered species endemic from the Portuguese Southwest coast and the only described Al hyperacumulator species of Plantaginaceae family. For this species do not exists databases of DNA sequences and there are no molecular studies described in literature.**

In the present study, we evaluated the stress induced by the presence of Al on *P. almogravensis*, using molecular markers (*ISSRs – Inter Single Sequence Repeats*). Five different genotypes of *P. almogravensis* were exposed, for 7 and 21 days, to 400 µM of Al and, the same genotypes, were maintained in control conditions (without Al). Ten ISSR primers were used to detect DNA damage in roots and leaves of the plantlets after 7 and 21 days. The presence and/or absence of DNA fragments in treated and non-treated samples was analised and the polymorphism rate was calculated for each genotype. On the average, comparing the control and the plants in stress conditions, we could verify that the roots were more affected than the leaves and, from the 7 to 21 days the polymorphism increased in the roots and was similar in the leaves. However, different behaviors were observed in the studied genotypes, some of them showing a decrease of the polymorphism ratio, from 7 to 21 days in culture, and others an increase. Our results suggest that some genotypes show tolerance mechanisms to stress conditions with the capacity to recover after prolonged Al exposure and that the roots were more affected than the leaves.

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