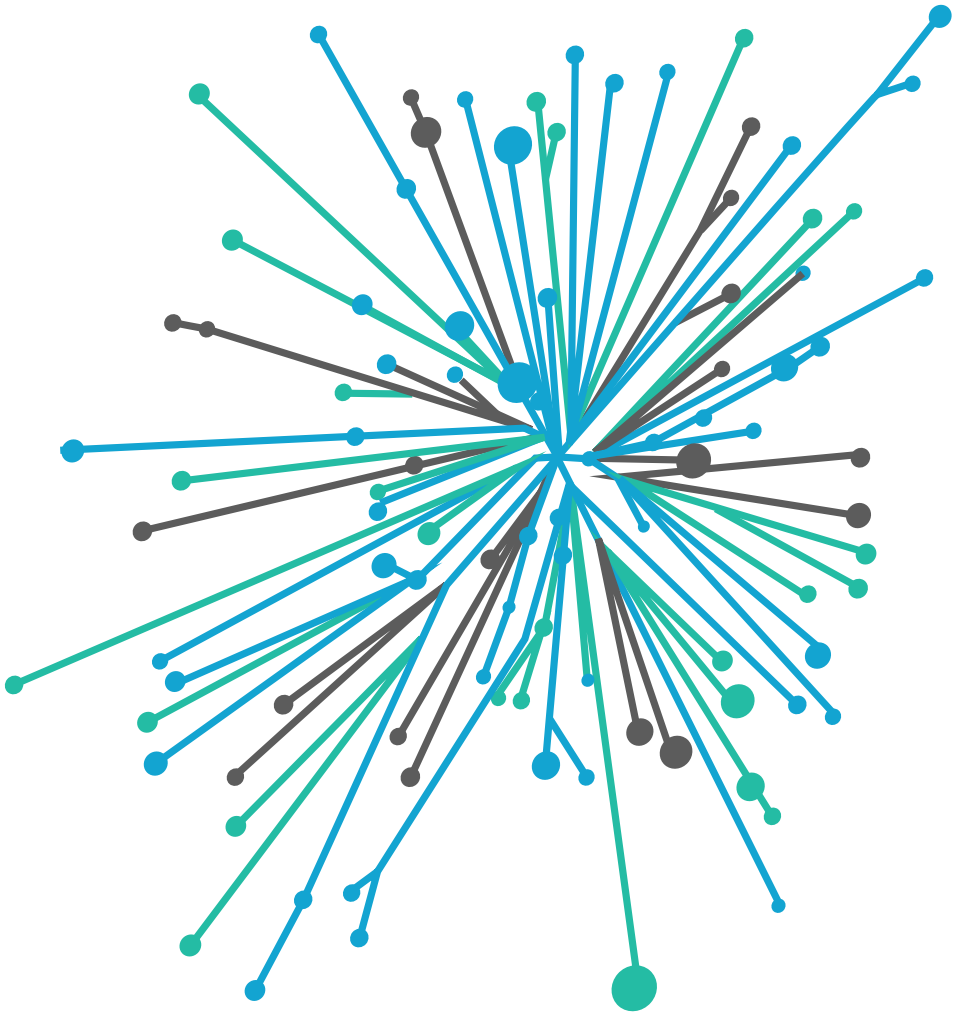
17th meeting

portugaliæ genetica

17 & 18 March 2016 | Porto



Organising & Scientific Committee:

Alexandra Lopes | i3S/ IPATIMUP
Miguel Arenas | i3S/ IPATIMUP
Nádia Pinto | i3S/ IPATIMUP

Invited Scientific Committee:

Carla Oliveira | i3S/ IPATIMUP
Filipe Pereira | CIIMAR

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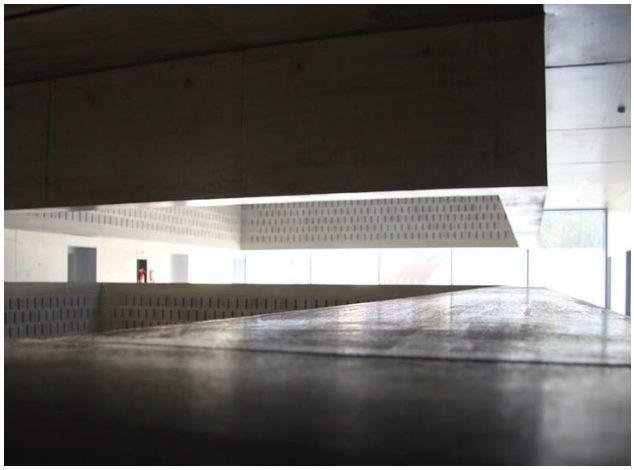
Venue & Location

i3s - Instituto de Investigação e Inovação em Saúde
Rua Alfredo Allen, 208 | 4200-135 Porto, Portugal



Important Informations

Presentations Instructions
The plenary sessions should last up to 45 minutes. Oral presentation should last up to 15 minutes.
Speakers presenting in the morning should hand in their presentations in the auditoriums until 9:00 of the presentation day. Speakers presenting in the afternoon sessions, should hand in their presentations during lunch break.
A data show and personal computer will be at the presenters' disposal. Technicians will be available to make sure that you have successfully submitted your presentation. You will be requested to provide your presentation in a USB key.

Poster Presentations
Posters should have 1.20m high and 0.90m wide, and will be presented on the designated poster area. Conference staff will be present to provide assistance. Authors should remain next to their poster during their poster sessions.

Poster Session I March 17 – poster 1-16
Poster Session II March 18 – poster 17-28
All posters must be placed before Poster Session I and remain in place until the end of Poster Session II.

Internet Access
Wireless Internet is freely available in the symposium venue.

Programme

Thursday, 17th March 2016

09.00 Registration

09.30 Opening session | Mário Barbosa | i3S Director

SESSION 01 | POPULATION GENOMICS AND EVOLUTION
Chair: António Amorim

09.45 **The history in our genes**
Cristian Cappelli | U. Oxford, United Kingdom

10.30 Coffee break

11.00 **On the importance of being structured: should we revisit the demographic history of species? (and how it could apply to humans)**
Lounès Chikhi | IGC, Lisbon, Portugal & CNRS, Toulouse, France

11.45 **Understanding the impact of chromosomal inversions on the evolution of the human genome**
João Alves | U. Vigo, Spain

12.15 **Chromosomal inversions and natural selection**
Jorge Vieira | i3S/ IBMC, Porto, Portugal

12.30 Poster session and Lunch

SESSION 02 | CANCER AND PATHOGEN EVOLUTION

Chair: Miguel Arenas

14.30 **Cancer phylogeography**
David Posada | U. Vigo, Spain

15.15 **Integrating NGS data to assess host-microbe interactions in asthma**
Marcos Pérez-Losada | George Washington University, USA & CIBIO-InBIO, Porto, Portugal

16.00 Coffee break

16.30 ***Escherichia coli* adaptation in the mammalian gut**
Isabel Gordo | IGC, Lisbon, Portugal

17.15 **Genome dynamics of a generalist microbe: competence for transformation generates stochastic variation and an open pangenome in *Bacillus subtilis***
Patrícia Brito | IGC, Lisbon, Portugal

20.00 Meeting Dinner

Friday, 18th March 2016

SESSION 03 | WIDENING THE SPECTRUM OF PATHOGENIC VARIATION

Chair: Carla Oliveira

09.30 **An analysis of the n=1 problem in human genetics**
Don Conrad | Washington University in St. Louis, USA

10.15 Coffee break

10.45 **Next-generation sequencing approaches in neurogenetics**
Isabel Alonso | i3S/ UniGene, Porto, Portugal

11.30 **Patterns of gene expression variation across dozens of human tissues and hundreds of individuals.**
Pedro Ferreira | i3S/ IPATIMUP, Porto, Portugal

12.15 **Sequence variation at *KLK* and *WFDC* clusters and its association to male infertility**
Patrícia Marques | i3S/ IPATIMUP, Porto, Portugal

12.30 Poster session and Lunch

SESSION 04 | EXPLORING THE REGULOME: FROM GENES TO PROTEINS

Chair: Alexandra Lopes

14.30 **Post-transcriptional control by multi-functional RNA-binding proteins: from phenotypes to mechanisms**
Nicola Gray | U. of Edinburgh, MRC Centre for Reproductive Health, United Kingdom

15.15 **How mRNA translation is involved in modulating nonsense-mediated decay in transcripts with AUG-proximal nonsense mutations**
Luísa Romão | INSA, Lisbon, Portugal

16.00 Coffee break

16.30 **Regulation of transcriptional reactivation of the oocyte during meiosis**
Rui Martinho | U. Algarve, Portugal

17.15 **Dissecting the molecular mechanisms controlling alternative polyadenylation in human T cells: *MCL1* 3'ends regulation**
Isabel Castro | i3S/ IBMC, Porto, Portugal

17.30 Poster prize & Closing Session

invited speakers

Speakers' Biographies

Cristian Capelli

U. Oxford, United Kingdom

Cristian Capelli obtained his PhD in Forensic Genetics at the Institute of Legal Medicine of the Univesità Cattolica del S. Cuore in Rome.
He has worked in the laboratories of Svante Paabo and David Goldstein and since 2006 he is an Associate Professor in Human Evolution at the Department of Zoology of the University of Oxford.
His research focuses on human evolution, with a particular attention to the processes (cultural and historical) that have shaped the distribution of genetic variation across human populations.
Current projects address the historical events that led to the peopling of the Mediterranean region and the European continent, and the pattern of migration and admixture in Sub-Saharan Africa.

Speakers' Abstracts

The history in our genes

Cristian Capelli

The distribution of genetic variation in human populations has been shown to be correlated with geography. However populations are not isolated from each other and gene-flow has occurred between groups located in proximity or separated by long distances. By analysing the genetic diversity of extant populations using genome-wide SNP data and haplotype-based approaches, we will explore how common admixture has been in the history of human populations, characterise episodes of gene-flow in Africa, America and Europe and reconstruct the ancestry profiles of admixed populations.

Speakers' Biographies

Lounès Chikhi

IGC, Lisbon, Portugal
& CNRS, Toulouse, France

Lounès Chikhi obtained his PhD in Population Genetics at Université Pierre et Marie Curie.
He is currently based at the Université Paul Sabatier in Toulouse, France, and also at the Instituto Gulbenkian de Ciência in Oeiras, Portugal, where he runs the Population and Conservation Genetics Group (PCG).
The PCG carries out research in the area of population genetics with a focus on conservation and human population genetics. In particular the group is interested in understanding the statistical properties of genetic data in natural or managed populations/breeds in order to determine when and how genetic data can be used to make statements about the populations' recent evolutionary history.
The group develops field and laboratory work together with data analysis and computer simulations. They have applied these methods to a wide range of species but in the last few years their main focus has been on endangered species from Madagascar and Borneo, and, for human populations, on the Neolithic transition.

Speakers' Abstracts

On the importance of being structured: should we revisit the demographic history of species ? (and how it could apply to humans)

Lounés Chikhi

Most species are structured and influenced by processes that either increased or reduced gene flow between populations over tens of thousands of years as a consequence of climate change, or recent anthropogenic actions. However, most population genetic inference methods assume panmixia (random mating, or complete mixing) across wide areas to make statements about population size changes. Under the assumption of panmixia these methods reconstruct a demographic history that is necessarily characterized by population size changes. This is potentially problematic since population structure has been shown to generate spurious signals of population size change. In other words, we can infer ancient population size changes that may have never occurred. Moreover, when the model assumed for demographic inference is mis-specified, genomic data will likely increase the precision of misleading if not meaningless parameters. For instance, if we use genomic data to date a bottleneck we may state that the population decreased by a factor 10 around 15,000 years ago with great "precision".
However, if the data were actually generated under a model of population structure, then the inference process under a bottleneck model might be meaningless. In addition, archaeological or climatic events around the bottleneck's timing might provide a reasonable but potentially misleading scenario. Genomic data may thus not necessarily lead to improved statistical inference. There is therefore a need to construct a much more solid inference framework allowing us to work in a context of model uncertainty (panmixia versus structure). During my talk I will present recent results obtained in the group in collaboration with the Institut de Mathématiques de Toulouse. I will argue that all (coalescent-based) methods claiming to identify population size changes do not infer population size changes. They infer a parameter that is equivalent to a population size only in panmictic models, or models with very high gene flow). I will show various cases where the inference is misleading and will apply our approach to human genomic data.
Finally, I may propose a temporary solution and discuss whether a longer-tem solution can exist.

Speakers' Biographies

João Alves

U. Vigo, Spain

João M. Alves graduated in Applied Biology in 2008 from the University of Minho, Portugal and in 2009 he was awarded with a research fellowship at the Instituto Gulbenkian de Ciência, Portugal.

He obtained his PhD in 2014 by the Institute of Biomedical Sciences (ICBAS), Porto, Portugal working on the evolutionary history of chromosomal rearrangements.

Currently he is a postdoctoral researcher at David Posada's group at the University of Vigo, Spain, working on NGS data analysis.

Speakers' Abstracts

Understanding the impact of chromosomal inversions on the evolution of the human genome

João Alves

The significance of genomic rearrangements (structural modifications that may involve loss or gain of genetic material) in evolution and their consequences in human health have been long recognized, in particular when involving large, cytogenetically detectable changes. However, their importance in genetic research has been overshadowed for decades in favor of smaller mutational changes of a different nature more amenable to be studied at the population level. Recently, advances in genome sequencing have revealed that one subtype of these rearrangements — chromosomal inversions — is far more common than previously admitted. At this stage, a very large number of inverted rearrangements have been detected and validated in the human genome, but such finding is difficult to reconcile with the classical interpretation of inversions as a common mechanism causing subfertility or even reproductive isolation (and ultimately leading to speciation). Moreover, despite the improved molecular and computational methods allowing an exponential increase in the discovery of chromosomal inversions, these rearrangements remain poorly characterized at the population level, and our understanding of its evolutionary impact is still largely limited.

Here, we make use of dense population genetic data to explore the evolutionary processes shaping two polymorphic inversions on the human genome, which have been subject to rigorous scientific scrutiny over the last years — *8p23-inv* and *17q21.31-inv*. Our results suggest that the genetic barrier created by these rearrangements facilitate the accumulation of sequence divergence over time, leading to significant changes in the recombination landscape of the affected genomic regions. Moreover, by targeting a large number of present-day populations, we showed that the spread of these rearrangements can result from complex past demographic changes (and distinct dispersal trajectories), without the need of invoking selection.

Altogether, our results highlight the role of these rearrangements as drivers of genome evolution (even at an intraspecific level) while providing relevant insights into the processes shaping the frequency and distribution of inversion polymorphisms in human populations.

Speakers' Biographies

David Posada

U. Vigo, Spain

David Posada obtained his PhD in Zoology in the Brigham Young University (Utah, USA). He has worked in the University of Oxford (UK), Variagenics Inc and MIT (Boston, USA) and then he got a Full Professor position at the Department of Biochemistry, Genetics and Immunology of the University of Vigo (Spain).

His research involves population genetics, phylogenetics and phylogenomics, evolutionary biology and bioinformatics. In particular, he is interested in theoretical, methodological and empirical aspects of the evolutionary analysis of genes and genomes. Currently, he is very interested in the evolutionary and population genetics analysis of cancer tumor cells, a topic where he recently obtained an ERC Consolidator Grant.

PHYLOCANCER: Cancer phylogeography

David Posada

The evolution of tumors in a body resembles the evolution of populations in more or less fragmented habitats. The tumor is usually an expanding population of clonal cells, which may differentiate to a bigger or lesser extent and disperse to contiguous or more distant tissues. During tumor progression, this population of cells is subject to distinct somatic evolutionary processes like mutation, drift, selection or migration, which can act at different points in time and geographical space.

Very recently, the discovery of extensive intratumor heterogeneity, together with the rise of single-cell genomics, has created an unique opportunity to study the phylogeography of cancer tumor cells. In the ERC project PHYLOCANCER we propose to exploit population genomics and phylogenetic concepts and methods to study the evolution of single cell-genomes from multiple regions within a tumor. We are studying two different types of cancer: colorectal cancer (CRC) and chronic lymphocytic leukemia. (CLL).
In this talk I will present the goals of the PHYLOCANCER project and its first, partial results.

Speakers' Biographies

Marcos Pérez-Losada

George Washington University, USA & CIBIO-InBIO, Porto, Portugal

Marcos Pérez-Losada obtained his PhD in Genetics in the University of Vigo (Spain).
He has worked in the University of Hull (UK), Brigham Young University (Utah, USA), GENOMA LLC (USA) and then he obtained an Assistant Research Professor position at both the George Washington University (USA) and CIBIO (Portugal).
His research involves invertebrate biodiversity, dynamics of human infectious diseases, bacterial genomics, HIV and host-microbe interactions, but also the evaluation of methods for phylogenetic and population genetic analysis and data types to study evolutionary processes at both the species and population levels.

Speakers' Abstracts

Integrating NGS data to assess host-microbe interactions in asthma

Marcos Pérez-Losada

Emerging next-generation sequencing (NGS) technologies have revolutionized the collection of genomic data for applications in bioforensics, biosurveillance, and for use in clinical settings. However, to make the most of these new data, new methodologies that can integrate in a computationally efficient manner large volumes of genomic data from different sources (e.g., DNA-seq, RNA-seq, 16S) need to be applied.

Our lab has developed a statistical framework called PathoScope to accurately and quickly analyze host and microbial NGS data for microbiome characterization (metagenomics and metataxonomics) and transcript differential expression (meta and transcriptomics). We routinely apply this and other analytical tools to the study of pulmonary diseases such as asthma in the Washington DC area (USA).

Asthma is one of the most common chronic respiratory disorders, currently affecting 17.7 million adults and 6.3 children in the US. The estimated total cost of asthma to society is ~$56 billions per year. The application of NGS technology has shown that microbial communities in the respiratory airways (i.e., the airway microbiome) play a significant role in the onset, development and severity of asthma.

However, little is known about their composition during health and disease, temporal dynamics (i.e., microbial succession), potential pathogens and how microbial communities may impact host function during disease. Here I introduce PathoScope and other analytical tools developed for the analysis of shotgun (DNA, RNA) and amplicon (16S) sequence data. Then I show a few examples of their application to the characterization of airway microbiotas, identification of pathogens and assessment of host-microbe interactions during asthma.

These studies have shown that: 1) airway microbiome composition and function change during asthma, 2) the airway microbiome is dynamic and its composition changes over time in asthmatics, 3) bacterial genera like *Moraxella*, *Staphylococcus*, and *Streptococcus* are significantly associated with chronic asthma, and 4) the airway microbiome may modulate host inflammatory and immune responses during disease.

Speakers' Biographies

Isabel Gordo

IGC, Lisbon, Portugal

Isabel Gordo obtained her PhD in Evolutionary Biology at the University of Edinburgh, UK working with Brian Charlesworth.
Currently she is the group leader of Evolutionary Biology group at Instituto Gulbenkian de Ciência in Oeiras, Portugal.
The lab combines both theoretical and empirical work with the aim of understanding the major forces that shape variation in populations.
They use *E. coli* as a model organism to test theoretical predictions about the genetics of adaptation in the context of environmental changes due to abiotic and biotic factors.
The main topics of current research are: evolution of antibiotic resistance caused by chromosomal mutations, adaptation to cells of the innate immune system, transition of commensalism to pathogenesis and evolution in the gut microbiota.

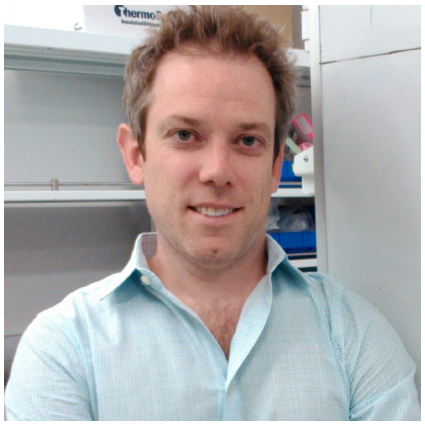
Speakers' Abstracts

Escherichia coli adaptation in the mammalian gut

Isabel Gordo

Understanding the dynamics and mechanisms underlying adaptive evolution is a principal aim in evolutionary genetics. Bacteria are excellent models organisms to study adaptation in real time under different selective pressures. *Escherichia coli* is a versatile species, both in genetic and phenotypic variation, that inhabits the intestinal track of most mammals and one of the first colonizers of the human gut. We have been following *E. coli* evolution as a commensal of the mammalian gut. Using mice as a host we find that in only one week of colonization many clones carrying different advantageous mutations emerge and compete for fixation in the mouse gut, a process known as clonal interference. Despite the high complexity of the natural environment studied the evolutionary process is highly reproducible as the same mutations occurr in populations of *E. coli* evolving in different mice. The results reveal a layer of complexity of the gut microbiota, which has been unknown so far, and demonstrate how rich and rapid the evolutionary dynamics of each bacteria inhabiting a healthy animal can be.

Speakers' Biographies



Don Conrad

Washington University in St. Louis, USA

Don Conrad completed his PhD with Jonathan Pritchard at University of Chicago, and a post-doc with Matthew Hurles at the Wellcome Trust Sanger Institute, before joining the Washington University in St. Louis as an assistant professor in 2011.
He is an active member of www.genomesunzipped.org, a group of professionals providing perspectives and tools for personal genomics. His main research interests are reproductive biology and the development of computational tools for genome analysis.
He is one of the founders of the GEMINI consortium, currently funded by the National Institutes of Health, which aims at identifying the genetic architecture of male infertility in populations of European ancestry.

Speakers' Abstracts

An analysis of the n=1 problem in human genetics

Don Conrad

The diagnosis of rare, idiopathic diseases is emerging as a primary application of medical genome sequencing. However, the application of standard tools from genetic epidemiology for many of these cases is frustrated by a combination of small sample sizes, genetic heterogeneity and the large number of singleton variants found by genome sequencing. In response, we have developed a statistical inference framework that is optimized for identifying unusual functional variation from a single genome, what we refer to as the "n-of-one" problem.

Using this approach we assess our ability to solve 1 million simulated cases of Mendelian disease, finding that 39% of disease genotypes can be identified as the most damaging unit in a typical exome background. We applied our approach to several cohorts of rare disease, including 129 n-of-one families from the NIH Undiagnosed Diseases Program, nominating 60% of 30 genotype or genotype pairs determined to be diagnostic by a standard clinical workup. Finally, we show that our approach provides a powerful way to include population databases in an integrative analysis by combining population sampling probabilities with gene expression data to improve the detection of UDP diagnostic genes. Our method provides well-calibrated p-values when applied to single genomes, and, with further work, could become a widely used epidemiological method like linkage analysis or GWAS.

Speakers' Biographies

Isabel Alonso

i3S/ UniGene, Porto, Portugal

Isabel Alonso obtained her PhD in Biomedical Sciences, at Institute de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, in 2006. During the PhD, she focused on identifying mutations in families with rare Spinocerebellar Ataxias (SCAs), as well as in the molecular and behavioural study of a mouse model with a major impact on the knowledge about these disorders and, in particular, on the role of calcium deregulation on cognitive function.
Following two years of postdoc she obtained a research position at Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal, under the Ciência 2008 program which allowed her to start independent research lines at UnIGENe and tackle scientific topics not being addressed, as the search for genetic modifiers by the study of patients and C. elegans models. Currently Isabel is an Assistant Researcher at i3S/ IBMC, Porto, Portugal.

Next-generation sequencing approaches in neurogenetics

Isabel Alonso

Neurodegenerative disorders are characterized by a gradually progressive and selective loss of anatomically or physiologically related neuronal systems and include, among others, the spinocerebellar ataxias, the hereditary spastic paraplegias and Parkinson's disease. The identification of the genes responsible for these conditions constitutes a critical step for their follow-up in daily clinical practice, genetic counselling and, ultimately, for the understanding of the molecular pathways resulting in neuronal dysfunction. During the last decades, the development of high-throughput technologies and computational approaches has transformed the study of genetic diseases. Next generation sequencing, in particular, proved to be a powerful tool for the study of Mendelian diseases. The aim of this presentation is to provide some examples of successful application of this approach to the study of the genetics of neurological diseases.

Speakers' Biographies

Pedro Ferreira

i3S/ IPATIMUP, Porto, Portugal

Pedro G. Ferreira completed his PhD in Artificial Intelligence from the University of Minho in November 2007. From 2008 to 2012, he was a Postdoctoral Fellow at the Bioinformatics and Genomics Laboratory, Center for Genomic Regulation (CRG), Barcelona.
During this period he worked extensively with next generation sequencing (NGS) data.
He collaborated with several groups in the CRG and the University Pompeu Fabra and several international consortia. In November 2012, he joined as a Postdoctoral Fellow the Functional Population Genomics and Genetics of Complex Traits group, School of Medicine, University of Geneva.
His research was focused on genomics for personalized health. During his two postdocs, he was or is involved in four major international consortia: ENCODE, ICGC-CLL, GEUVADIS and GTEx.
In August 2014, he joined the start-up company Coimbra Genomics as a senior bioinformatics specialist. He worked in the design and development of clinical decision support systems for personalized medicine.
In May 2015 he joined the Expression Regulation in Cancer group at IPATIMUP as an FCT Investigator 'Starting grant'. His main research focus is the development of information systems to interpret personal genomics data for clinical diagnosis and precision medicine.

Speakers' Abstracts

Patterns of gene expression variation in human populations across tissues and individuals

Pedro Ferreira

Gene expression is a key determinant of cellular phenotype, and many complex human diseases are caused by changes in gene expression and splicing. Genome-wide RNA expression analysis has long been a mainstay of genomics and biomedical research, and is being obtained increasingly often through RNA sequencing. This data is accumulating across tissues, individuals and species, and it is providing insights in to gene expression changes underlying human disease. However, there has been limited data to investigate systematically how gene expression and splicing varies across tissues and individuals within human populations. The Genotype-Tissue Expression project (GTEx) is providing a comprehensive atlas of gene expression across multiple human 'non-diseased' tissues sampled from recently deceased human donors. In the pilot phase, samples from up to 46 distinct body sites were RNA sequenced, comprising ~1700 samples from 175 individuals.
In this talk I will discuss some of our results in trying to characterize how transcriptional patterns vary both across tissues and across individuals within the scope of GTEx and other large scale sequencing projects. Tissues exhibit characteristic transcriptional signatures that show stability in post-mortem samples. These signatures are dominated by a relatively small number of genes, though few are exclusive to a particular tissue, and vary more across tissues than individuals. Genes exhibiting high inter-individual expression variation include disease candidates associated with sex, ethnicity and age. Primary transcription is the major driver of cellular specificity, with splicing playing a secondary role; except for the brain, which exhibits a characteristic splicing program. Variation in splicing, in contrast, despite its stochasticity, may play a comparatively greater role in defining individual phenotypes. Changes in splicing may have an important phenotypic impact, even in the absence of changes in overall gene expression. I will also discuss results on the detection of splicing variation between different groups of individuals and how those changes may be associated to genetic variation.

Speakers' Biographies

Nicola Gray

U. of Edinburgh, United Kingdom

Nicola Gray completed her PhD at the European Molecular Biology Laboratory in Heidelberg and was awarded a PhD by the University of Glasgow.
She then carried out postdoctoral research at the University of Wisconsin, Madison, where she developed an interest in translational regulation during early development.
In 2000 she became a Group Leader at the MRC Human Genetics Unit in Edinburgh and in 2008 she moved her laboratory to the MRC Human Reproductive Sciences Unit where she remains associated with the University of Edinburgh.

Speakers' Abstracts

Post-transcriptional control by multi-functional RNA-binding proteins: from phenotypes to mechanisms

Nicola Gray

It has become evident that mRNA translation and stability are key points in regulating gene expression and that their dysregulation is involved in a wide spectrum of diseases (e.g. neurological, metabolic, oncogenic and reproductive disorders). Yet our understanding of how these processes are controlled and linked remains in its infancy. However, it is clear that RNA-binding proteins play pivotal roles in regulating mRNA translation and stability, although their roles and mechanisms of action are only delineated in a small number of cases.

Germ cells and early embryos have provided many important insights into this area, in part as they undergo periods of transcriptional quiescence meaning that changes in protein synthesis have to be achieved by the activation, repression or destruction of pre-existing mRNAs. Classically germ cell mRNAs are stored with a short poly(A) tail, are translationally activated by cytoplasmic polyadenylation (i.e. addition of a newly synthesised tail) and can be silenced by deadenylation (removal of the poly(A) tail): Such changes in poly(A) tail status are under strict temporal control with timing being distinct for different classes of mRNAs. However, it is also clear that other mechanisms to regulate the utilisation of germ cell mRNAs must exist.

Reduced fertility affects 10-15 % of couples world-wide, although the genetic basis for this is poorly understood in most cases. However, data from model organisms underscore the importance of correctly regulating mRNA translation or stability. In particular, we are interested in a germ cell specific RNA-binding protein family whose founding member, Deleted in Azoospermia (*DAZ*), was identified as a candidate gene for male infertility, as it is frequently deleted in men with little or no sperm (Azoospermia). Genetic studies of other family members (DAZ-like [*DAZL*] and *Boule*) in multiple model organisms have established the critical role of this family in male and female fertility, and also in the oocyte-zygote transition in mice.

Our work, which has focused on *DAZL*, has revealed that it can bind to the 3' untranslated regions of a subset of essential germ cell mRNAs to activate their translation. We also find that *DAZ* and *Boule* are mRNA-specific translational activators, suggesting that this represents a conserved role, which, at least in part, explains their critical functions in gametogenesis. Importantly, we have

shown that it does not do this by promoting cytoplasmic polyadenylation but through a distinct mechanism which involves recruitment of poly(A)-binding protein (PABP), which normally binds to the poly(A) tail, through a direct RNA-independent interaction.
In addition, we have found that DAZL is multi-functional, as it also inhibits the deadenylation of mRNAs with long poly(A) tails, which may maintain such target mRNAs in a translationally active state. Since mRNA translation and deadenylation are often thought to be intimately linked, with PABP having functions in both processes, we have investigated the relationship between these DAZL activities, and provide mechanistic insights which reveal they are separable. Lastly, we explore whether *DAZL* mutations in patients with reduced fertility affect these functions. Thus, our data provide insight into fundamental mechanisms by which germ cell mRNAs are regulated to enable gametogenesis, and into the poorly understood links between mRNA translation and deadenylation.

Speakers' Biographies

Luísa Romão

INSA, Lisbon, Portugal

Luísa Romão developed research work as a graduate student in the University of Pennsylvania, School of Medicine, Philadelphia USA and obtained a PhD in Molecular Genetics by the Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.
She is currently the Head of the mRNA Metabolism Group of the Human Genetics Department at Instituto Nacional de Saúde Doutor Ricardo Jorge and Invited Professor at Faculdade de Ciências of Universidade de Lisboa (FCUL). She has been involved in studying mechanisms of post-transcriptional control of gene expression in health and disease. Half of the research in the laboratory focuses on nonsense-mediated mRNA decay (NMD), which likely evolved to safeguard cells from potentially deleterious proteins produced as a consequence of routine mistakes in gene expression. Her data has been crucial to clarify the interplay between mRNA translation, PTC definition and NMD, in order to understand the mechanisms of PTC-associated diseases. The other half of her research focuses on the molecular basis of the control of protein synthesis in human cells and its importance in diseases such as cancer, diabetes, and neurological diseases.

Speakers' Abstracts

How mRNA translation is involved in modulating nonsense-mediated decay in transcripts with AUG-proximal nonsense mutations

Luísa Romão

Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that recognizes and selectively degrades mRNAs carrying premature termination codons (PTCs). In addition, several studies have also implicated NMD in the regulation of steady-state levels of physiological mRNAs, and examples of natural NMD targets are transcripts containing upstream short open reading frames or long 3' untranslated regions.
The strength of the NMD response appears to reflect multiple determinants on a target mRNA. We have reported that human mRNAs with a PTC in close proximity to the translation initiation codon (AUG-proximal PTC), and thus, with a short open reading frame, can substantially escape NMD. Our data support a model in which cytoplasmic poly(A)-binding protein 1 (PABPC1) is brought into close proximity with an AUG-proximal PTC *via* interactions with the translation initiation complexes. This proximity of PABPC1 to the AUG-proximal PTC allows PABPC1 to interact with eRF3 with a consequent enhancement of the release reaction and repression of the NMD response. Here, we provide strong evidence that the eIF3 is involved in delivering eIF4G-associated PABPC1 into the vicinity of the AUG-proximal PTC. In addition, we dissect the biochemical interactions of the eIF3 subunits in bridging PABPC1/eIF4G complex to the 40S ribosomal subunit. Together, our data provide a framework for understanding the mechanistic details of PTC definition and translation initiation.

Speakers' Biographies

Rui Martinho

U. Algarve, Portugal

Rui Martinho obtained his PhD in Biology at the University of Sussex, UK. He did a postdoc in the laboratory of Ruth Lehmann, HHMI-NYU Medical Center, USA and from 2003 to 2006 he was an associate in the Howard Hughes Medical Institute unit at New York University School of Medicine (New york, USA).
Fom 2006 to 2011 he was an independent investigator at Instituto Gulbenkian de Ciência (Oeiras, Portugal) and in 2011 became an Assistant Professor at University of Algarve, Portugal. His main research interest is to take advantage of *Drosophila melanogaster* as a model system to study germ-line development, tissue morphogenesis and cell division during development.

Since tumorigenesis is frequently associated with loss of tissue integrity and misregulation of cell proliferation, this work is expected to give a contribution into a better understanding of molecular and cellular processes associated with the onset and development of cancer.
He also leads a private research initiative – Thelial Technologies (Screen for novel cancer chemotherapy drugs using *Drosophila melanogaster* as a model system).

Speakers' Abstracts

Early programming of the oocyte epigenome temporally controls late prophase I transcription and chromatin remodeling

Rui Martinho

Oocytes are arrested for long periods of time in the prophase of the first meiotic division (prophase I).
As chromosome condensation poses significant constraints to gene expression, the mechanisms regulating transcriptional activity in the prophase I-arrested oocyte are still not entirely clear. We hypothesized that gene expression during the prophase I arrest is primarily epigenetically-regulated.
We have defined for the first time the *Drosophila* female germ line epigenome and found that the oocyte has a unique and remarkably diversified epigenome characterized by a simultaneous enrichment in euchromatic and heterochromatic marks. We observed that the perturbation of the oocyte's epigenome in early oogenesis resulted in the temporal deregulation of transcription prior to meiotic maturation and critically affected female fertility.
Taken together, our results indicate that the early programming of the oocyte epigenome primes meiotic chromatin for subsequent functions in late prophase I.

oral communi-cations

01 | Oral Communication

Chromosomal inversions and natural selection

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Chromosomal inversions are common in many groups of animals, despite the expected reduction in fertility due to the production of nonfunctional gametes during meiosis, in individuals heterozygous for such rearrangements. In *Drosophila*, they are widespread because recombination is generally suppressed in males, and in females aberrant recombinant products preferentially contribute to the polar body nurse cells. Therefore, in the *Drosophila* genus the expected reduction in fertility is not so obvious. This group of species is thus ideal to study the mechanisms that are involved in the generation of inversions, increase in frequency (and eventual fixation), as well as their impact on the genome. Species of the virilis phylad are an excellent model to address these issues, because closely related species show contrasting patterns regarding inversion polymorphism. In this work, we sequenced the genome of a *D. americana* strain from the south of the distribution, as well as the genome of one *D. novamexicana* strain, which allowed us to characterize at the molecular level the breakpoints of almost all *D. americana* polymorphic inversions. In addition, we obtained pool-seq data for one *D. americana* population from the north, centre and south of the distribution, to characterize the impact of chromosomal rearrangements at the genome level.

This work was financially supported by national funds through FCT - Fundação para a Ciência e a Tecnologia/MEC - Ministério da Educação e Ciência and when applicable co-funded by FEDER funds within the Partnership Agreement PT2020 related with the research unit number 4293.

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Genome dynamics of a generalist microbe: competence for transformation generates stochastic variation and an open pangenome in *Bacillus subtilis*

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Bacillus subtilis is a natural competent bacterium that can grow in a diverse set of environments that span the invertebrate and the vertebrate gut, as well as several plant-associated, soil, and marine niches. In this study we quantify its genomic diversity and infer for the first time the evolutionary processes that can explain the high level of diversity observed. We analyzed 43 whole genomes (ten lab strains and 33 natural strains, including two new gut strains). A phylogenetic analysis of 685 core genes indicates five major branches of diversification within *B. subtilis* and two independent origins of all laboratory strains. Overall we recover a remarkable genomic diversity that translates into a conserved core genome of approximately 1500 genes and a variable, open, pangenome of approximately 7900 genes. This diversity is due to a large proportion of low frequency genes that are acquired from closely related species. In wild isolated overall gene acquisitions are counterbalanced by equivalent rates of gene losses, explaining why cloud genome (41% of the species pangenome) represents only a small proportion of each genome. Our results are highly suggestive of a competence-driven essentially stochastic process of gene acquisition where the long-term maintenance of acquired genes in the genomic repertoire of the species dependents on local and global fitness effects. We propose that this process allows *B. subtilis* to acquire heterologous DNA that can confer a selective advantage when changing through environments and ecological interactions. Our results strongly emphasize the generalist character of *B. subtilis*, which can occupy a wide range of ecological niches and cycle through them.

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03 | Oral Communication

Sequence variation at *KLK* and *WFDC* clusters and its association to male infertility

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Infertility is a major reproductive health issue currently estimated to affect about 186 million people worldwide, in which male factors either alone or in combination with female causes contribute to the disease etiology. Despite the many significant advances achieved over the last decades in this field, namely in the identification of major and minor genetic abnormalities leading to disease, to date male infertility continues largely unexplained and a high proportion of cases are frequently reported as idiopathic. Proteolysis-related genes with key roles in the cascade of semen liquefaction such as the kallikireins (*KLKs*) located at chromosome 19q13.3-13.4, and the whey acidic protein four-disulfide core domain (*WFDC*) and their neighbors' semenogelin 1 and 2 (*SEMGs*) positioned at chromosome 20q13 are excellent candidates to seek for additional genetic susceptibility factors to male infertility. Thus, not only the sperm are coated by EPPIN, a protease inhibitor of the WFDC family, but also, upon ejaculation, they are entrapped in the coagulum, a cross-linked matrix established by SEMGs. Later on, the coagulum is hydrolyzed, mainly by proteases KLK3 and KLK2, in a fine-tuned process regulated by other KLKs that allows a motility gain by the sperm. To access the impact of *KLK* and *WFDC* sequence variation into male infertility, we first performed a pilot screening of both coding and non-coding regions covering 220 segments and ~93 kb of the human genome by a massive parallel sequencing approach in a pooled sample strategy comprising 143 cases and 75 controls.

Afterwards, the most promising candidates were surveyed individually in a cohort of 238 cases and 217 controls and several semen samples were subjected to mass spectrometry assays for variant peptide profiling. Overall, these analyses revealed a higher burden of low-frequency variants with potential deleterious effects in cases than in controls but solely for the group of *KLK* genes (32 vs. 17; P=0.0106). Additionally, we validated 11 variants overrepresented among infertility cases, including 3 substitutions found to cluster in the KLK binding pocket, 2 missense mutations overlapping in the KLK three-dimensional structure and 2 additional variants mapping in consecutive residues of N-terminal region. Consistent with this, as far as we could evaluate from semen peptide profiling, candidate variants appear more prone to affect KLK activity rather than its protein levels. Other variants identified in our study consisted of 3 missense mutations predicted to modify SEMG cleavage patterns and possibly affecting spermatozoa motility, as well as a copy number variation in *SEMG1* and a common nucleotide substitution in *KLK7* found to be associated to a reduced risk of the disease. In conclusion, our results provide evidence for a contribution of *KLKs* and *SEMGs* variability into the loss of semen quality in a process mediated by anomalous semen liquefaction which seems to affect male reproductive success.

04 | Oral Communication

Dissecting the molecular mechanisms controlling alternative polyadenylation in human T cells: MCL1 3'ends regulation

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Approximately 70% of all mammalian genes produce multiple mRNAs with distinct 3'ends due to the selection of different polyadenylation signals (PAS) in the 3'UTR (1). This mechanism, known as alternative polyadenylation (APA), is a mode of co-transcriptional gene regulation, fundamental for mRNAs stability, translation and/or localization (2). In the immune system, it was shown that upon T cell activation there is a global switch in PAS selection from distal to proximal PAS, originating mRNAs with shorter 3'UTRs and, consequently, less prone to regulation by miRNA and RNA-binding proteins (RBPs) (3). However, despite of its significant role in gene expression, the molecular mechanisms underlying APA events remain unclear.

Here, we identified molecular players involved in *MCL1* APA in human T cells, providing new insight into this mechanism. Mcl-1 (Myeloid Cell Leukemia 1) is an anti-apoptotic protein essential for embryogenesis and for the development and maintenance of T cells (4). We identified three *MCL1* mRNA isoforms resulting from APA in the 3' UTR, by 3'RACE mapping. We showed that upon T cell activation there is an enrichment of the shorter mRNA isoforms, correspondent to an increase in Mcl-1 protein levels. Additionally, we demonstrated that shorter 3'UTRs lead to higher levels of luciferase protein than longer 3'UTRs, revealing a function for MCL1 shorter 3'UTRs in protein production. Using luciferase assays and western blot, we additionally show that miR-17 specifically targets the *MCL1* longest mRNA, contributing to the downregulation of this mRNA isoform upon T cell activation. To identify APA regulators of MCL1, we made a lentivirus-based shRNA screen to knockdown polyadenylation factors, RBPs and factors involved in transcription. Surprisingly, we identified INTS9 (Integrator complex subunit 9), which has a function in small nuclear RNAs (snRNAs) 3' end processing (5), as a regulator of *MCL1* APA. Furthermore, by RNA-immunoprecipitation (RIP) we showed that INTS9 directly binds to the *MCL1* mRNA. This is the first study identifying INTS9 as an APA regulator and linking Integrator to mRNA 3' end processing. Overall, our results provide mechanistic insight in APA regulation occurring during T cell activation.

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01 | Poster

Mutation and recombination drives HIV-1 *in vitro* fitness recovery

Miguel Arenas[1,2,3], Ramon Lorenzo-Redondo[4,5] and Cecilio Lopez-Galindez[4]

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The understanding of the evolutionary processes underlying HIV-1 fitness recovery is crucial for HIV-1 pathogenesis, antiretroviral treatment and vaccine design. It is known that HIV-1 can present large mutation and recombination rates, however the specific contribution of these evolutionary forces to the "*in vitro*" viral fitness recovery has not been simultaneously quantified. Here we analyzed substitution, recombination and molecular adaptation rates in a variety of HIV-1 biological clones derived from a viral isolate after severe population bottlenecks and a number of large population cell culture passages. We found a significant relationship between the fitness increase and the appearance and fixation of mutations. In addition, the fixed mutations presented molecular signatures of positive selection through the accumulation of non-synonymous substitutions. Interestingly, viral recombination correlated with fitness recovery in most of studied viral quasispecies. The genetic diversity generated by these evolutionary processes was positively correlated with the viral fitness. We conclude that HIV-1 fitness recovery can be derived from the genetic heterogeneity generated through both mutation and recombination, and under diversifying molecular adaptation. The findings also suggest nonrandom evolutionary pathways for *in vitro* fitness recovery.

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How relevant can the mother be?

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Kinship testing, using unlinked autosomal markers, is the most common routine in genetic forensics. However, there are some distinct genealogies that belong to the same kinship class (genealogical relationships that have the same allele sharing probabilities), being then theoretically indistinguishable. Indeed, the probabilities of a specific genetic configuration assuming different pedigrees from the same autosomal kinship class are equal and, therefore, the likelihood ratios weighing the hypotheses are 1 [1-4]. The most common example, described in the literature, of genealogies sharing the same kinship class are half-siblings, avuncular and grandparent-grandchild pedigrees. The aim of this work was, using unlinked autosomal markers, test if the analysis of the mother of one or both of the available individuals influences this categorization, allowing the distinction (theoretical, at least) of the pedigrees. Likelihood ratios comparing the different hypotheses: half-siblings vs. avuncular, half-siblings vs. grandparent-grandchild and avuncular vs. grandparent-grandchild, were computed using the software Familias v 1.97, assuming that (a.) the mother of one of the individuals, and (b.) the both mothers, are available for testing. The results showed that adding the tested child's mother it is not relevant for the distinction between the three pedigrees remaining these on the same kinship class. Nevertheless, if the unquestioned child's mother (or both) is considered, the possibilities half-siblings vs. avuncular and avuncular vs. grandparent-grandchild are always pondered differently, being the comparison half-siblings vs. grandparent-grandchild differentiable only under particular genotypic conditions.

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03 | Poster

A set of conserved PCR primers for the study of seven plant families

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The number of plant species currently identified is greater than 300,000. The use of molecular genetic methods to study such diversity of species often depends on the targeting of conserved genomic regions. However, it is difficult to accommodate in a single target region all genetic variation existent in divergent plant lineages. It is therefore necessary to identify conserved regions suitable for lower taxonomic levels in order to increase the success of DNA-based investigations. We have designed conserved PCR primers for amplification of four chloroplast DNA (cpDNA) regions (atpF-atpH, psbA-trnH, trnL CD and trnL GH) in seven plant families: *Asteraceae*, *Brassicaceae*, *Iridaceae*, *Orchidaceae*, *Poaceae*, *Rosaceae* and *Salicaceae*. The primers were designed using 28 multiple sequence alignments with over 11,000 reference sequences from all available species of each plant family. The relevance of the conserved regions was tested by PCR in DNA extracted from plant leaves. Our PCR tests were able to amplify all target regions in representative samples from the seven families. The conserved genomic regions and PCR primers can be used in diverse areas of plant research, including species identification, DNA barcoding, ecology, metagenomics, phylogeography or phylogeny.

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04 | Poster

Detection of Hepatitis B virus using multiplex PCR

Adriana Resende [1,2], Ana Constança[3], Elizabeth Pádua[4], Isabel Pereira-Castro[2], Amanda Bradley Stewart [5], Rory N. Gunson[5], Helena Ramos[3] and Filipe Pereira[1,2]

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The hepatitis B virus (HBV) is a significant threat to public health worldwide, making it necessary to improve clinical diagnosis and extend epidemiological studies. Diagnostic methods based on nucleic acid testing are more sensitive than traditional serological testing and allow the direct viral identification. However, the design of efficient nucleic acid-based assays is challenged by the high genetic diversity characteristic of viruses. Consequently, methods that rely on the analysis of one or two genomic regions for the viral identification are more susceptible to produce false negative results due to sequence variation at primer or probe binding sites. Here we describe multiplex PCR assay for the amplification of HBV genomic regions. The assay was designed to base all identifications on the analysis of multiple regions, reducing the likelihood of falsenegative results due to polymorphisms in primer-binding sites. The selected regions were first validated by singleplex PCR and then combined in multiplex reactions. No false negative results were observed, *i.e.*, all tested samples yielded positive amplifications. The assay detected the viral infection in samples with low viral loads and no cross-reactivity were observed with other viruses. Overall, this assay can be used to study HBV with high specificity and robustness using low cost laboratory equipment. The genetic markers selected here could also be used in other assays or sequenced for population or evolutionary studies.

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05 | Poster

The players of the collaborative non-self recognition Malus self-incompatibility system

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In flowering plants, a widespread mechanism to prevent inbreeding is gametophytic self-incompatibility (GSI), where the self-incompatible phenotype of the pollen is determined by its haploid genome. In Solanaceae, Plantaginaceae, Rubiaceae, and Rosaceae species pistil specificity is determined by an extracellular ribonuclease, called S-RNase. The male determinant, always a F-box gene, can be determined by one gene, as in the self recognition *Prunus* (Rosaceae) system, or multiple genes, as in the non-self recognitions *Pyreae* (Rosaceae; called *SFBBs*) and Solanaceae (called *SLFs*) systems. In the collaborative non-self recognition system, each of the multiple S-pollen genes interacts with only a subset of its non-self S-RNases, and none of them can interact with their respective self-S-RNase. Therefore, in such systems, a large number of S-pollen genes must exist in a single S-haplotype. Presently, in Pyreae 18 genes have been identified using both PCR approach using primers for conserved regions from genomic DNA and BAC libraries. To identify the most complete set of Malus x domestica *SFBB* genes, we used a de novo RNA-seq approach to analyze the pollen transcriptomes of 10 S-haplotypes, as well as the young leaf, ovary, stigma, style, sepal, petal transcriptomes of Golden delicious (S2S3). High diversity and/or deletion of *SFBB* genes as predicting targets of non self-S-RNases are not a common mechanism of recognition in M. domestica, as recently proposed in Solanaceae.

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06 | Poster

A deep characterization of Y-chromosome J haplogroup lineages in Portugal

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Y-chromosome haplogroup J is assumed to have originated in the Middle East, region from where populations suffered a major expansion during the Neolithic period, migrating towards almost every direction including North Africa, Southern Europe and the Eastern routes from the Caucasus far as the Indian subcontinent (1). Those population movements resulted in the scattering of haplogroup J in the whole peri-Mediterranean region, reaching Portugal, located at the westernmost dispersion extreme but with a relatively lower impact (1)(2). Haplogroup J has two main branches: J1-M267 and J2-M172. Thought to have originated roughly around 10.000 years ago, during the Neolithic period, presenting, nowadays, slightly differences in their distributions. However, until now, downstream sub-haplogroup diversity within the J1-M267 and/or J2-M172 clades has not been assessed in the general Portuguese population (2)(3). In order to select the most informative J sub-haplogroups for studding the Portuguese population, a deep literature search on previously described subclades was made taking into account their global spatial distribution (2). This would lead to select a total of seventeen SNPs defining different subclades (1)(2)(3)(4), whose location in the Y-chromosome phylogenetic tree was thoroughly checked. The next step was to design the genotyping strategy which was based in a SNaPshot approach relying in two multiplex amplifications i) subclades within J1 (including the main J polymorphism – J-M304) with eight polymorphisms and ii) subclades within J2, with nine polymorphisms. The developed approach will allow the fine characterization of J lineages in the Portuguese population, which expectedly will turn possible to discriminate in time and space the origin of the different migration waves responsible to the introduction of distinct J sub-types in Portugal. Furthermore, the data obtained will contribute to enrich the already existing databases on Y-chromosome lineages, facilitating useful information for future analysis.

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Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases (preliminary study)

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Sexual assault biological evidence are among the most difficult samples types encountered in forensic laboratories. Y-STR markers constitute a valuable tool in the analysis of these traces. Yfiler® Plus PCR Amplification Kit and AmpFLSTR® Yfiler® PCR Amplification Kit constitute two different approaches that can be used on this analysis. The aim of this project is to compare the success rate of these two kits. A total of 325 trace samples from forensic casework were quantified and analyzed. Yfiler® Plus PCR Amplification Kit presented a greater robustness and sensitivity, especially on samples with higher female:male DNA ratio. This kit presented better results in 43% of all samples compared to the 5% obtained with AmpFLSTR® Yfiler® PCR Amplification Kit. Furthermore, Yfiler®Plus PCR Amplification Kit amplified a higher number of samples than AmpFLSTR® Yfiler® PCR Amplification Kit (127 and 97, respectively). It was concluded that Yfiler® Plus PCR Amplification Kit constitutes an excellent alternative to AmpFLSTR® Yfiler® PCR Amplification Kit and a good option to implement in forensic laboratories.

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08 | Poster

Characterization of autosomal AIM-indels in the Philippines: population data and structure analysis

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The Philippines is an archipelago located in Southeast Asia, which is divided in the regions of Luzon, Visayas and Mindanao. It is characterized by a cultural, linguistic and ethnic diversity, currently constituted by over 170 ethno-linguistic groups. This study aimed to the genetic characterization of autosomal ancestry informative markers (AIMs) in the population of the Philippines. A total of 381 samples from the three main regions of the country were genotyped through a multiplex system comprising 46 AIM-INDELs. The analysis performed at intrapopulation level indicated the absence of a significant substructure among the three regions, thus allowing the establishment of a single genetic database for the INDEL set. Further worldwide structure analysis using HGDP-CEPH and other published data for the same markers showed that the Philippines have an essentially East Asian ancestry and a close genetic similarity to populations in this region, especially with Taiwan. In conclusion, the results indicate that this multiplex system of 46 AIM-INDELs is highly efficient to infer the biogeographical ancestry of individuals, thus proving a powerful tool in population genetics, as well as in specific forensic scenarios.

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Identification of transgenic fishes using multiplex PCR

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Increasing consumer demand for fish products and the resulting overfishing is leading to the saturation of the fish production through traditional aquaculture. This problem has prompted the construction of transgenic or genetically modified (GM) fishes with foreign DNA artificially inserted into their genomes, in particular the growth hormone (GH) gene used to increase growth and feed conversion efficiency. In recent years, GH-transgenic strains have been developed in a number of species, including common carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) or Atlantic salmon (*Salmo salar*), among others. The Food and Drug Administration (FDA) has already approved the commercialization of Aquadvantage salmon on 2015, the first GM animal on the market for human consumption. Therefore, strict regulatory approvals will be implemented for molecular methods capable of detecting the foreign DNA used to make a GM species. However, no DNA-based method for the simultaneous detection of different transgenic fishes is currently available. Here we describe the development of a molecular kit based on multiplex polymerase chain reaction (PCR) for the detection of the transgenic elements (i.e., promoters and coding regions of genes) used to produce GM-fishes. PCR primers were designed to amplify sections of the GH gene of five species (Atlantic Salmon, Rainbow Trout, Common Carp, Grass Carp and Nile Tilapia) and three gene promoters (Ocean Pout AFP promoter, Common carp and Grass Carp ß–actin promoters) commonly used in GM-fishes, so that each element yields a different length (from 80 to 269 bp). All target regions were initially tested by singleplex PCR using DNA extracted from reference samples. The target regions were successfully amplified by multiplex PCR and discriminated by conventional and capillary electrophoresis. Overall, the method presented here allows the detection of the transgenic elements used in GM-fishes most likely to be on the market in the near future.

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Evaluation and Internal validation of a retrotransposons quantification and amplification kits (InnoQuant™ HY and InnoTyper™ 21) for DNA analysis of degraded biological evidence and rootless hair

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Forensic laboratories frequently receive, from crime scene investigators, biologic samples that are very limited in terms of DNA quantity and quality, due to environmental stresses, storage time and preservation conditions. In an attempt to facilitate the analysis of this type of biological samples, InnoGenomics Technologies developed new commercial kits for DNA quantification and amplification (InnoQuant™ HY and InnoTyper™ 21) that use mobile DNA elements (retrotransposons) as molecular markers. The aim of this work is to perform the internal validation of InnoQuantTM HY and InnoTyperTM 21 kits, under the working conditions of the Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ). The results obtained with these new kits were compared with the results obtained with the kits currently in use at the same lab, which uses STRs as molecular markers (Quantifiler® Duo and GlobalFiler™). For the validation of these new kits, tests of sensitivity, repeatability, reproducibility, minimum threshold/contamination and mixtures were performed. The selected samples, from extracts of LPC-PJ caseworks, were mainly hairs (with follicle); but also blood, contact traces, bone fragments, and teeth. The selection criterium was the inclusion of profiles with possible degraded DNA and profiles without results or with inconclusive results.

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All the experimental work of this project were implemented with the support of InnoGenomics Technologies, the company responsible for the study, development and commercialization of InnoQuant™ HY and InnoTyper™ 21 kits.
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Y-STRs in a population sample from east Europe immigrants

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Short tandem repeat (STR) are the class of genetic polymorphisms most commonly used in forensic due to easy and rapid PCR amplification, ability to analyze many STRs in one reaction (multiplex), high discrimination power and relatively high mutation rates which confers them a high variability. They can be studied both in autosomes and in sex chromosomes. These markers are used in kinship genotyping and trace analysis, both for individual identification and for population studies. For this last purpose lineage markers, such as mitochondrial DNA, chromosome X and Y markers, have particular forensic utility. They are also very useful to study cases that involve degraded samples, human remains identification and sexual assault cases.

Regardless of autosomal STRs are much more informative than Y- STRs, their study helps to supplement the autosomal information. They are very useful in mixtures where excess female DNA is present allowing us to get a male profile. However a match between an evidentiary sample and a suspect means that male relatives (father, brothers, sons, etc.) are also not excluded as potential sources of the evidence. They are also highly helpful tools to infer population histories, discover genealogical relationships, for tracing human migration and evolution through male lineage.

Ukraine is a country in Eastern Europe, populated by 44.6 million people, which borders with the Russia Federation, Belarus, Poland, Slovakia, Hungary, Romania and Moldova. Since the study of Y-STRs on the Ukrainian population is not very deepen, we decide to study a sample of east Europe immigrant individuals (n=27), to check if there are some similarities between the Portuguese population and the Ukrainian population, since the Ukrainians are currently the second largest foreign community residing in Portugal.

In this work we present the haplotype frequencies from this population sample and we compare the obtained results with our population (north of Portugal).

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Considerations on the use of a set of 15 STRs for analyses of questioned half- and full-sibship

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With the growing of the use of DNA as a tool for human identification, it is also increasing the need of studies to provide data for the analysis of relationships with different degrees of kinship. For other pedigrees than identity and paternity, the absence of share of alleles at one locus does not exclude the biological relationship, since individuals do not necessarily inherit the same ancestral alleles. Thus, these kinship investigations are more difficult to solve also because the majority of literature data on kinship testing is reduced to paternity and data on other genetic links are scarce. In this work we intend to provide statistics for the kinship analyses: (a.) full-siblings vs. unrelated, and (b.) half-siblings vs. unrelated, resorting to real pairs of related (and unrelated) individuals typed for a commercial kit of 15 autosomal STRs (Short Tandem Repeat markers). We considered 170 real pairs of full-siblings and 74 of half-siblings and, correspondingly, the same number of pairs of unrelated individuals, typed with the STR multiplex kit AmpFlSTR® Identifiler™ as data source. The statistical evaluation was computed through likelihood ratios (LR), comparing the probability of the genotypic configuration of the pairs of full-siblings assuming full-sibship, with the one obtained assuming unrelatedness. The same was calculated for the set of unrelated individuals under the same assumptions of relatedness. The same procedure was reproduce for half-siblings and unrelated pairs under the assumptions of kinship: half-sibship and unrelatedness. For 97% of the total set of pairs of full-siblings the real kinship was the one pointed as the most likely, as well as for 99.4% of the total set of unrelated pairs. Under the framework of half-sibship for 86.5% of the real pairs of half-siblings the favoured kinship was the real, as well as for 89.2% of the cases of pairs of unrelated individuals. Despite some powerful LR values, the results suggest that a higher number of markers should be used to obtain strong and statistically robust results for this kinship analysis.

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Collection methods of semen stains in textiles, with PCR inhibitors, for DNA analysis

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In criminal cases involving sexual assaults, the presence of semen, as well as analysis of DNA profiles present, are important not only to prove sexual contact, but also to identify the perpetrator. If the evidences are contained in textile materials (e.g. cloths, bed linen, etc.) it must be taken into account that the dyes used in their manufacture (e.g. indigo dye, a known PCR inhibitor present in denim) can cause problems in DNA analysis. PCR inhibitors can complicate amplification by interfering with DNA polymerase and its cofactors or by directly interacting with the DNA and primers. Their presence can lead to total allele loss on STRs analysis and to false negative results, especially in low quality/quantity of DNA samples. The removal or concentration reduction of these inhibitors prior to PCR amplification is critical for genotyping success. The most common technique used to overcome this problem is the dilution of the DNA sample in order to minimize the concentration of inhibitor. However, this technique is not suitable for degraded or low-template DNA samples. Thus, the improvement of collection techniques may be the next step to increase the success of genotyping samples that contain inhibitors. The methods of collecting in tissue include: (1) cut the fabric in the area of interest; (2) use a wet cotton swab, rubbing the tip on the trace; and (3) repeatedly press an adhesive on the fabric. In forensic samples, where the quality/quantity of DNA is usually small, it is essential that the method of collection is as efficient as possible. Thus, this review aims to highlight the importance of creating a standardized protocol for collecting samples in tissue, pointing out some advantages and disadvantages of the methods, in order to prevent loss of critical samples and improve the efficiency of DNA analysis.

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Review: Swabbing techniques for semen collection in sexual assault cases

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Sexual assaults (SA) are currently one of the most prevalent crimes in our society and affect mostly women. In these cases DNA evidence is frequently obtained through semen collection and has become a crucial part in their resolution, since the presence of semen in SA cases indicates sexual contact and can identify the aggressor. Consequently, the most relevant evidence analysed in SA investigations are the vaginal and anal swabs obtained during the medical exam. Forensic samples from SA cases are frequently degraded or contain little amount of DNA for analysis due to the time lag between the assault and the performance of the medical exam. These samples commonly cause problems during the amplification phase (PCR) of the analysis, resulting in stochastic effects such as allele drop-out or severe peak imbalance, among others. Many studies have addressed the problem of low-template DNA samples in order to increase the number of detected alleles (e.g. increase number of cycles during PCR, post-PCR purification), however these methods could also result in stochastic effects that complicate data interpretation. As a result, some authors have suggested that improving the collection method may be the most reliable way to obtain the best DNA profiles. There are several swabbing techniques for sample collection from the victim and it is left to the medical professional which one to use. These methods include the "classic" single swab, two simultaneous swabs and the most recent double swabbing technique. This review intends to compare the above-mentioned techniques, exposing differences between them and referring some advantages and disadvantages in order to emphasize the importance of a standardized protocol for sample collection that allows the procurement of the best possible samples, resulting in less cases of secondary victimization.

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15 | Poster

How a polymorphic pseudogene is born: insights from *GBA3*

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Evidence has been provided that the process of pseudogenization can be adaptive [1, 2], but the extent to which that occurs is still elusive. Polymorphic pseudogenes [3] are clearly well suited to address the issue, since they have simultaneously functional and pseudogenic alleles segregating in populations, providing the opportunity to observe a pseudogenization event in progress. In humans, *GBA3* is one of the known polymorphic pseudogenes, whose functional allele encodes the cytosolic -glucosidase, an enzyme that might play a role in the detoxification of plant glycosidases [4]. Its pseudogeneized allele was originated by a substitution (rs358231) creating a premature stop codon that disrupts enzyme activity [5], being currently commonly found among non-African populations (~14%). Here, to explore the process of pseudogenization at *GBA3*, we have retrieved sequences from the 1000 Genomes Project in order to search for signs of selection in several human populations. Our results show quite opposite trends in functional and non-functional sequences: Tajima's D values were positive for the sequences carrying the pseudogenized allele. Interestingly, the analysis of dN/dS ratios obtained for the phylogenetic tree of vertebrates showed that even though *GBA3* is overall under strong selective constraints (dN/dS = 0.21) in primates it is neutrally evolving. Inspection of the *GBA3* gene tree further revealed a synonymous substitution (p.Arg354Cys) with a striking distribution since the ancestral variant attained moderate frequencies ([Symbol]15%) in African populations while being almost absent in non-African ones. Given the proposed role of *GBA3*, we hypothesised that this gene had lost its functional relevance in populations not depending on the consumption of plants containing xenobiotic compounds.

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Ribosomal Protein S4 paralogues in mammalian spermatogenesis: X-gene compensation?

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Ribosomal protein S4e is a highly conserved protein present in all kingdoms. The *RPS4* gene is found on autosomes in all vertebrates except mammals, in which it is X-linked, having also retained an ancestral Y-linked copy in some lineages. In primates the ancestral *RPS4Y* gene duplicated and originated a second Y-linked copy (*RPS4Y2*) in Old World Monkeys, while in the mouse *RPS4X* acquired an intronless paralogue on chromosome 6 encoding a highly identical protein (*Rps4l*). The primate-specific *RPS4Y2*, as well as mouse *Rps4l*, are expressed during spermatogenesis and may compensate for the transcriptional silencing of the X-linked copy during meiotic sex chromosome inactivation. In this study we aim to characterize lineage-specific potentially functional copies of RPS4 in mammals. We have identified S4l orthologs in three rodent families (*Muridea*, *Cricetidae* and *Spalacidae*) indicating that the duplication occurred more than 43.9 MY ago. Rodent S4l duplicates present low dN/dS ratios, suggesting functional conservation. Furthermore, by exploiting the available draft genome sequences of several mammalian species we have found RPS4 autosomal retrogenes with conserved open reading frames in dog, cow, horse and rabbit. Interestingly, in those species which retained a Y-linked *RPS4* gene (pig and cat) we did not succeed in finding potentially functional duplicates in other chromosomes.

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Is the mitochondrial DNA common deletion a candidate biomarker for spinocerebellar ataxia type 3?

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD; MIM#109150; ORPHA98757) is an late-onset proteinopathy, corresponding to the worldwide most prevalent spinocerebellar ataxia. Abnormal conformation of mutated ataxin-3 promotes a toxic gain of function, compromising several cellular mechanisms, namely mitochondrial function. Mitochondrial DNA (mtDNA) depletion as well as increased number of deletions (namely the m.8470_13446del4977, also known as "common deletion", an age-related marker) have been associated with neurodegeneration. For SCA3 such alterations have been previously studied only in a cell model and in a few patients' blood samples. Aiming to evaluate these findings in a larger and independent set of patients, as well as to test the potential of mtDNA content and the common deletion as biomarkers of disease progression, we measured the levels of mitochondrial encoded NADH dehydrogenase 1 (MT-ND1), NADH dehydrogenase 4 (MT-ND4) and ribonuclease P RNA component H1 (RPPH1) by quantitative real-time PCR (qPCR). Blood samples from 93 SCA3 patients, 16 asymptomatic carriers and 103 controls were analysed. The mtDNA content was estimated as the mitochondrial genome to nuclear genome ratio (MT-ND1/RPPH1); the common deletion was estimated as the ratio between the two mitochondrial genes (MT-ND4/MT-ND1). No differences between mtDNA content in SCA3 mutation carriers (patients and asymptomatics) and controls were found. The common deletion was significantly more frequent in patients (1.8 times higher) and asymptomatic carriers (4.2 times higher) than in controls. No significant correlations between mtDNA content or common deletion with disease duration, as well as with age at onset and CAG repeats size in expanded allele were detected. Results from this study suggest the common deletion may be a potential biomarker for SCA3, but this needs replication in an independent cohort of patients to confirm its value as a biomarker. Furthermore, a more detailed investigation of the association between the frequency of the common deletion and clinical features is necessary.

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A new method to detect the formation of non-B DNA structures *in vitro*

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DNA can adopt more than ten types of DNA structures that are different from the classic Watson and Crick B DNA helix, collectively known as non-B DNA conformations(1). Recent studies have revealed that many types of non-B DNA conformations play important roles in recombination, DNA replication, transcription and genetic instability(2) (3). Despite the importance of non-B DNA in genetic diseases and DNA metabolic processes, mechanisms by which genomic instability occurs remain largely unde¿ned. It is therefore important to develop reliable experimental systems to elucidate the mechanisms of non-B DNA formation and instability. Here we describe a new method to detect the presence of non-B DNA structures *in vitro*. The new assay uses two complementary oligonucleotides, designed to include a putative non-B DNA conformation. After inducing the annealing of the oligonucleotides, the resulting duplex DNA construct is subjected to structure-sensitive enzymatic cleavage (for example, the S1 nuclease to cleave unpaired nucleotides at the hairpin loops(4)). The formation of the non-B DNA structure is detected by the absence of PCR amplification of the digested duplex DNA construct. We tested our new approach using three constructs with the hairpins and part of the stem of a 93-nt cloverleaf-like structure (named structure A) previously identified by us in the human mitochondrial DNA (mtDNA)(5)(6). Our results demonstrate that the structure A hairpins occur *in vitro*, supporting the possible formation of this structure *in vivo*. These results also prove the utility of our method to detect the formation of non-B DNA structures, which can be used to study other alternative DNA conformations.

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A next-generation sequencing approach for the simultaneous study of Wilms tumors and parathyroid tumours

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Wilms Tumor (WT), also known as nephroblastoma, is a rare kidney condition which affects 1 in ~10.000 infants and children. This tumor is of complex etiology with underlying causes still incompletely understood. The majority of known mutations in *WT* are of somatic origin, with approximately one-third of patients displaying mutations in *WT1*, *CTNNB1*, *AMER1* (*WTX*) and/or *TP53* genes. In contrast, familial predisposition mutations are very rare. Recently, we used Next-Generation Sequencing (NGS) technology and a customized amplicon panel (TruSeq Custom Amplicon, Illumina) to detect somatic mutations of *WT1*, *AMER1*, *TP53* and *CTNNB1* (exon 4) genes, and identified mutations in 11 of 36 patients (30.5%). These results prompted us to design a new sequencing panel which incorporates a larger set of genes and addresses the problems encountered with low or inexistent coverage for some amplicons. In addition to those 4 genes, the new panel also includes genes involved in the SIX1/SIX2 pathway, which are frequently mutated in WT patients with blastemal-type histology(1), microRNA-processing genes (*DROSHA*, *DICER1*, *DGCR8*, *XPO5* and *TARBP2*), which were found to be mutated in over 10% of WT patients(2), as well as other genes (e.g., *MYCN*) previously known to be involved in Wilms tumorigenesis. Moreover, we included 6 other genes (*CDC73*, *CTR9*, *PAF1*, *LEO1*, *RTF1* and *WDR61*), which encode subunits of the PAF1 complex. Among these genes, CTR9 is already known to be a WT-predisposition gene(3), and *CDC73* is associated with the pathogenesis of the hereditary hyperparathyroidism-jaw tumour syndrome, and is also frequently mutated in sporadic parathyroid carcinomas. Using this new sequencing approach it will be possible to sequence genes involved in different types of familial cancer syndromes and sporadic tumours, simultaneously. Moreover, this strategy may also lead to identification of novel mutations of PAF1 complex genes, not yet known to be implicated in WT and parathyroid tumour development.

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Polo/Polo-like kinase-1, a candidate regulator of RNA Polymerase II

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Polo is a conserved Ser/Thr cell cycle kinase [1] that regulates its own protein levels by alternative polyadenylation (APA) via an auto-regulatory feedback loop mechanism: Polo overexpression induces a switch from a distal to a proximal polyadenylation signal in the 3'UTR (pA2 to pA1) resulting in a decrease in protein levels [2]. Plk-1 (Polo-like kinase 1) has been identified in proteomic analyses of RNAPII-associated chromatin [3]. As polyadenylation is co-transcriptional, we hypothesize that Polo's function in APA may arise from its kinase activity through phosphorylation of Ser/Thr residues present in RNA binding proteins or in the carboxy-terminal domain (CTD) of the RNA polymerase II (RNAPII). We performed ChIP analysis on 24h embryos using the polo1 (kinase-dead) and polo9 (null) heterozygous mutant strains and observed an increase in RNAPII levels along the polo gene in comparison to the wild type strain. By western blot, we observed a decrease in the phosphorylation levels of CTD Ser-5 and CTD Ser-7 in polo9 mutants in comparison to the wild type, suggesting that Polo may directly or indirectly affect CTD phosphorylation levels. A function for Polo/Plk-1 on RNAPII modifications and chromatin occupancy will be discussed.

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Are pure normal FMR1 alleles originated from contractions of expanded repeats?

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Fragile X syndrome (FXS) is the most common known cause of inherited intellectual disability. In most cases, FXS is caused by an expansion over 200 CGGs in the 5'UTR of the *FMR1* gene (Xq27.3). The hypermethylation of the full mutation causes gene silencing, hence the absence of the fragile X mental retardation protein. Based on repeat size and instability, other *FMR1* alleles can be designated as normal (stable 5-40 CGGs), intermediate (slightly unstable 41-54 CGGs) and premutated (very unstable 55-200 CGGs).

During FXS diagnostic routine in our lab, we identified four mosaic males caring a full mutation and a normal allele at *FMR1*. Evidence of four extremely rare independent post-zygote contractions of the full mutation to the normal range led us to first hypothesize the existence of a predisposing haplotype to large contractions. Also, we questioned whether other normal (CGG)n alleles found in the general population could have been originated through contractions of full expansions.

We analyzed 127 unrelated FXS patients (including the mosaics) and 212 healthy males. Allele sizes and interruption patterns have been assessed by repeated-primed PCR followed by capillary electrophoresis. Next, we genotyped 3 SNPs within the same haplotypic block as the CGG repeat to identify stable *FMR1* lineages of all normal and expanded alleles. A further comparison of haplotypes including 4 fast-evolving markers (*DXS998*, *DXS548*, *FRAXAC1* and *FRAXAC2*) within each lineage allowed us to reconstruct phylogenetic networks, crucial to visualize genetic distances among analyzed *FMR1* alleles.

All normal repeats of the mosaic FXS cases were pure CGGs, with no AGG interruptions. We have not found a single SNP background shared by all, but the two mosaics from the TTT lineage did share the same extended STR haplotype A21: 34-44-38-336, suggesting the existence of a lineage-specific instability factor. We next tested whether other non-interrupted repeats observed in our control population have resulted by the same process of contraction from the expanded range or, alternatively, evolved from other

normal alleles by losing interruptions. In our cohort of healthy controls, we have found 8 normal pure alleles, 5 of them from the TTT lineage. Networks showed these alleles to be phylogenetically close to normal interrupted alleles, supporting their origin more frequently by the loss of one AGG.
In conclusion, the pool of normal FMR1 alleles includes CGG repeats evolving within the normal range at a regular mutation rate over thousands of years, but also alleles arisen recently by large contractions of full mutations. The study of repeat instability underlying these two groups of normal pure (CGG)n alleles would be of utmost importance for the research on both molecular mechanisms and clinical aspects of the X-Fragile syndrome

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Genetic variation of *ATXN3L*, *JOSD1* and *JOSD2* in Machado-Joseph disease patients

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Much attention has been given to the ataxin-3 gene after the identification of an expanded (CAG)n tract, responsible for Machado-Joseph disease (MJD). MJD is a dominant late-onset neurodegenerative disorder mainly characterized by progressive cerebellar dysfunction but highly pleomorphic. The size of the expanded allele explains only part of the age-at-onset (AO) variability highlighting the importance of other modifiers. The normal *ATXN3* allele was one of the first AO modifiers to be suggested. In fact, several lines of evidence support this and propose a partial loss-of-function mechanism on MJD pathogenesis: a protective role of wild-type ATXN3 in MJD neurotoxicity has been observed in a fly model [1], patients homozygous for the expansion present a more severe phenotype [2], and an enhanced stress response is observed in ataxin-3 knockout models [3]. Besides *ATXN3*, three other cysteine proteases belong to the smallest family of deubiquitinating proteins (DUBs) - the MJD class: ataxin-3 like (*ATXN3L*; Xp22.2) and josephin domain-containing proteins 1 (JOSD1; 22q13.1) and 2 (*JOSD2*; 19q13.33). The proved DUB activity and residue similarity in the catalytic triad of all members of this subfamily led us to hypothesize that they may compensate for the decreased levels of wild-type ATXN3 observed in MJD by exerting similar functions. We searched for genetic variants in the regulatory and exonic regions of *ATXN3L*, *JOSD1* and *JOSD2* to assess their possible role as MJD modifiers.

We optimized multiplex reactions to amplify and sequence the regions of interest. In our cohort of 39 MJD patients analyzed so far, the expansion size accounted for 59% of the AO variation. We found 3 variants in ATXN3L: NG_021356.1:g.6404G>A, NP_001129467.1:p.Gly332Asp, and NG_021356.1:g.6680A>C. The position showing a non-synonymous change in ATXN3L may be a non-conserved residue since two other amino acid changes have been reported in this position in *ATXN3*. Three other SNPs have been detected in our cohort: NM_014876.5:c.6T>C and NP_055691.1:p.Ser48Arg in *JOSD1*, and NM_138334.3:c.*39G>A in the 3'UTR of *JOSD2*. Interestingly, two of the patients presenting higher differences between observed and expected AO (according to our data) were found to be heterozygous for two variants. The finding that the 3'UTR variant in *ATXN3L* was observed in a case with 10 yrs earlier AO and the p.Ser48Arg (JOSD1) identified in a 13 yrs later AO suggests that both risk and protective MJD modifiers may

have been detected in our candidate genes.
To complete this part of the project, we will extend the variant screening to a larger cohort of patients and the most promising variants following statistical analyses will be selected for functional assays in order to elucidate the underlying mechanisms that may explain their influence on MJD pathogenesis.

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Influence of repetitive elements on copy number variants associated with X-linked intellectual disability

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Copy number variants (CNVs) are structural genomic variants (insertions or deletions), ranging from 1 kb to several Mb in length. They can be classified as either copy number polymorphisms (CNPs), which exist in multiple allelic states, or rare copy number variations which are often associated with genomic disorders (Cooper *et al*, 2009; Henrichsen *et al*, 2009).
Among those regarded as pathogenic, there are insertions and deletions across the X-chromosome that confer susceptibility to X-linked intellectual disability (XLID) (Whibley *et al*, 2010). Repetitive genomic elements (e.g., LINEs, SINEs, LTR elements, etc.) have been shown to be catalysts of CNVs (Brown *et al*, 2011), contributing to their instability (Hastings *et al*, 2010; Chen *et al*, 2014) and subsequent genomic rearrangement. Therefore, estimating the frequency of these elements on XLID-related CNVs might lead to a better understanding of the underlying molecular mechanisms and might also help to correlate their involvement in XLID.
Here, we performed the characterization of the breakpoints of pathogenic XLID-related CNVs followed by a comparative analysis between pathogenic and population CNVs on the X-chromosome. As such, features such as size and type of structural variation of XLID-related CNVs as well as number and type of repetitive elements in their breakpoints were considered.
As expected (Veerapa *et al*, 2015), CNV gains and duplications are more frequent (67%) than losses and deletions (33%). Moreover, most of the variants tend to be either small

to moderate in size (1-100 kb) or extra-large (1-155 Mbp), which suggests that CNV size is not an important factor in potentiating pathogenicity. Most of the pathogenic CNVs are proximal to population CNVs (1-50 kb apart) and some population variants (Sudmant *et al*, 2015) intersect or are within the structural aberrations, thus underlining the complex nature of CNVs.

Regarding the relative frequency of repetitive elements, LINEs (30%) and SINEs (21%) constitute the majority of repeats nearby pathogenic breakpoints. Overall, L1, Alu, simple repeats and LINE2 account for 69%, 65%, 56% and 52% of the total repeats, respectively. LTR elements only account for 16% of all elements.

Therefore, the relative abundance of active retrotransposons such as L1 and Alu in a 1-kb radius from pathogenic breakpoints might explain the molecular basis underlying the events that lead to structural aberrations related to X-linked intellectual disability.

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Translational dynamics of germ cell development

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Mammalian spermatogenesis is a bizarre developmental process that takes weeks to complete, and results in the genomic copy number of germ cells shifting from 2N to 4N to 1N. It has been proposed that translational control is an important regulatory mechanism during this process- whereby transcripts are produced and then translated days apart. The purpose, the extent and timing of this translational control, and the factors involved, are relatively unknown. Deep sequencing of ribosome-protected mRNA fragments provides a new way of studying translational control, but the application of this method to the study of spermatogenesis has been hindered by the requirement of high quantity of starting material. In this work we have generated Ribosome Profiling data of the murine wild-type testis in order to investigate the complex regulatory network controlling spermatogenesis. A typical experiment produces over 44M reads, which, when coupled with cycloheximide treatment, results in 1,100 reads/kb in 5' UTRs, compared to 50 reads/kb in gene bodies and 7 reads/kb in 3' UTRs. Interestingly, 30% of reads map greater than 10kb away from annotated genes, suggesting that some of the pervasive transcription observed in spermatogenesis may be functional. Finally, we discuss our attempts to optimize the preparation of NGS libraries to low concentrations of ribosome-protected fragments. This approach reduces the quantity of cells/tissue required to obtain translatome data and can potentially be applied to isolated germ cell-types of different stages of spermatogenesis.

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Updating the study of pharmacogenetic polymorphisms in the Portuguese Gypsies

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Polymorphisms in genes coding for drug-metabolizing enzymes (DME) are major players in interindividual variability in drug response. Among DME, cytochrome P450 family 2, subfamily D, polypeptide 6, CYP2D6, is one of the most important, because it is involved in metabolism of 25% to 30% of all prescribed drugs, including antidepressants, antipsychotics, anti-arrhythmics, ß-blockers, cancer chemotherapeutics, among others (1,3).
The enzymatic activity of CYP2D6 varies widely among individuals, mainly due to functional genetic variations at the encoding gene, resulting in significant clinical consequences for drug metabolism and individual risk of adverse events or drug efficacy (4).
The *CYP2D6* gene is highly polymorphic, containing many allelic variants that confer altered enzymatic activity (the Human Cytochrome P450 Nomenclature Committee (www.cypalleles.ki.se/3), whose distribution across population also differs widely.
The Gypsies represent an interesting population case study that is still understudied in many aspects. During the Gypsy diaspora, group fragmentation into smaller communities occurred often, while maintaining the traditional endogamous practices. From a genetic point of view, this has led to genetic drift effects and reduced genetic diversity (5), whose impact in the fraction of diversity of pharmacogenetic relevance only recently began to be addressed in the community from Portugal (3). This justifies the study of *CYP2D6* in this population, as well as in the Portuguese host population, to find how much they differ from one another.
Viewing that, eleven SNPs in *CYP2D6*, before associated with the most common alleles accounting for decreased enzymatic function, were selected to be examined through a strategy based on Long Range PCR and subsquent Mutiplex Single Base Extension (SNaPshotTM reaction).

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Polymorphic compensatory mutations as phenotypic modifiers

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Genetic diseases with an apparently simple Mendelian inheritance can express a phenotype with reduced severity or even fail to express the disease at all, a phenomenon that is known as “incomplete penetrance”. One of the contributing factors is the co-occurrence of polymorphic variants that may compensate the deleterious effect of the disease-causing mutation. The revision of well-established cases of mutation-compensation pairs revealed some interesting general findings. The distance between the mutation and the polymorphism is variable among the different compensatory pairs witch may indicate a minor influence of the distance in the compensatory process. Another important outcome is the fact that a single polymorphism can compensate multiple mutations, rescuing the healthy phenotype. In addition, we were able to find examples of compensations that require more than one polymorphic site to be functionally rescued. The frequency for each mutation and variant was collected from the 1000Genomes Project, co-existence of the pathological mutation and the co-inherited polymorphism was observed in some individuals allowing the prediction that some individuals carry naturally compensated genetic background which is a finding of paramount importance in medical genetics and genetic counselling.

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In silico survey of NAPRT methylation across different cancer types

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Nicotinamide adenine dinucleotide (NAD) acts as a cofactor in redox reactions and as a substrate for NAD-consuming enzymes, such as Sirtuins and PARPs, which are involved in important cell functions from gene expression regulation to DNA repair, cell signaling and apoptosis. The role of NAD-dependent signaling and the high energy demands for cancer cell proliferation turns NAD metabolism into an important target for cancer biology and treatment. For example, inhibitors of nicotinamide phosphoribosyltransferase (Nampt), the main NAD synthesis enzyme from nicotinamide, have reached clinical trial stages, and the expression of nicotinate phosphoribosyltransferase (*Naprt*) is an important biomarker in the use of nicotinic acid as a co-adjuvant in Nampt inhibition treatments. In previous work [1], we showed that both genes are differentially expressed between normal tissues and cancer cell lines. Here, we present *in silico* results from the analysis of Cancer Genome Atlas (TCGA) data, focusing mainly on *NAPRT* methylation and its relation to gene expression. We used the TCGA data portal to download the Infinium HumanMethylation450 BeadChip data for selected cohorts. Methylation levels were obtained as beta-values for 14 different probes assigned to *NAPRT* gene. A control dataset was included in the analysis. We also used available online tools (cBioPortal, Broad GDAC Firehose), to evaluate methylation, mRNA expression, copy number variations, as well as their correlations in specific datasets. The methylation profile in different *NAPRT* gene regions showed that probes located in the CpG island in promoter and first exons of the gene were mostly unmethylated. The cg15537850 probe located outside of the CpG island, in the 1500bp region upstream the TSS, presented methylation beta-values above the average value. In the particular case of brain cancer (glioblastoma and low grade glioma) we observed low *NAPRT* expression and a moderate negative relationship was found between those probes and gene expression mean levels. The cg15537850 located upstream the CpG island had the strongest correlation. Two of the probes located in gene body were considered hypermethylated and the probe located in the 3'UTR was also methylated, however, these patterns do not differ between the control dataset and cancer samples. It has been proposed that methylation of exons modulates alternative splicing [2] and, considering the alternative *NAPRT* transcripts previously described by us [1], where the skipping of exon 5 was present, we hypothesize that methylation could be involved in the regulation of this event. Due to the predictive use of *NAPRT* expression levels for tailored therapeutics with Nampt inhibitors, further analyses are required to establish the link between *NAPRT* methylation, expression and alternative splicing both in normal tissues and cancer.

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Clinical Paradigms and Pharmacogenomics: The Tamoxifen Portuguese case study

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This work investigates the impact of genetic testing for *CYP2D6* in relation to tamoxifen in the management of women with breast cancer within Portuguese hospital context. Portugal has no implemented measures based on pharmacogenomics analysis prior to therapy, functioning as a cultural sample control when analysing the individual and economic factors present in clinical practice paradigms. Through qualitative/ quantitative studies regarding the impact of pharmacogenomics in breast cancer and personal interviews to management board and/or decision makers from major oncological centres. Data yielded contradictory reasons for common adoption of pharmacogenomics practice, both based in economic factors and cultural/clinical bias. Specific cultural and /or clinical bias was identified and we propose viable courses of action able to exert change in cultural medical habits.

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