Portugal, it represents frequencies of about 4%; however, their main subclades were poorly addressed in Portuguese samples until now. In present study, we examined a set of unrelated men from mainland Portugal for the most known E-M78 sublineages and respective internal microsatellite diversity.
**Methodology:** A total of 17 individuals, previously typed for the M78 marker (12 from central and five from northern regions of mainland Portugal), were analyzed for the three main E-M78 sublineages V12, V13, and V22. Genotyping was made by PCR-RFLP or Sanger sequencing using primers previously described. The internal variation of E-M78 samples was evaluated by the analysis of seven Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393) using primer sequences obtained at https://strbase-archive.nist.gov/ystr_fact.htm. Multiplex PCR reactions were carried out using the QIAGEN Multiplex PCR kit (Qiagen) with the forward primers labelled on the 5' end and separation of DNA fragments in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).
**Results:** Most individuals belong to the V13 subclade (n = 9) (52.9%). Two individuals belong to the V12 subclade (11.7%) and one to the V22 subclade (5.9%). Five individuals (29.4%) were assigned to the paragroup M78*. The seven analyzed Y-STRs defined a set of 13 different haplotypes in the Portuguese sample set. We found no haplotypes shared by different subclades.
**Discussion:** We highlighted the most common sublineages in a set of M78-derived Y-chromosomes of Portuguese individuals. Most E-M78 chromosomes (about 53%) belong to the E-V13 subclade, similar to results found in other European populations1. The E-V13 subclade was suggested to have a post-Neolithic expansion into Europe from the Near East, where it originated, via the Balkans. The E-M78 subhaplogroups E-V12 and E-V22, also found in the Portuguese population, could was involved in trans-Mediterranean migrations directly from northern Africa, probably during the Islamic period in the territory. The Y-STR analysis encompasses a collection of different haplotypes in the Portuguese samples, suggesting the probability of different evolutionary histories for the E-M78 chromosomes in the country.
**Reference**
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## P2 - NON-CANONICAL SYNTHESIS OF UPF1 PROTEIN CONTRIBUTES TO ITS ONCOGENIC ROLE IN COLORECTAL CANCER

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Colorectal cancer (CRC) is the third leading cause worldwide and projections point towards an increase over the next two decades. Gene expression dysregulation of several genes involved in CRC contribute to disease development.
The up-frameshift 1 (UPF1) protein plays important roles in several cellular mechanisms and acts as a tumour suppressor in most cancers. However, in CRC, this protein has been described as working as an oncogenic protein. In order to understand the molecular mechanisms underlying the oncogenic role of UPF1 in CRC, we have analysed mRNA and protein levels in different types of cancer. In silico analyses have shown that UPF1 is overexpressed in CRC and lung cancer compared to the other analysed cancers. Also, UPF1 expression is significantly greater in CRC than in normal tissues. Experimentally, we observed that UPF1 expression is maintained under stress conditions that compromise global protein synthesis. In this regard, we tested whether UPF1 translation initiation can be mediated through an alternative cap-independent mechanism. We showed that the 5' untranslated region (UTR) of UPF1 transcript allows cap-independent translation initiation and mapped the minimal sequence required for this mechanism to work. This region also mediates translation initiation in transcripts lacking a cap structure and under stress conditions like endoplasmic reticulum stress, hypoxia and mTOR pathway inhibition. Then, we designed antisense RNA oligonucleotides (ASOs) that target the minimal region and observed a reduced expression of UPF1 in CRC cells treated with those ASOs compared to cells treated with control ASOs.
All in all, these results show that alternative translation initiation mediated through UPF1 5'UTR allows UPF1 expression levels to be maintained under conditions observed in the tumour microenvironment, which globally repress protein synthesis. Thus, ASOs targeting the minimal region responsible for allowing UPF1 expression can be the beginning of a new RNA-based therapy to prevent CRC development.

## P3 - DILATED CARDIOMYOPATHY WITH A DOUBLE GENETIC DIAGNOSIS IN ARRHYTHMOGENIC-RELATED GENES

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We report a complex case of a family with a clinical diagnosis of dilated cardiomyopathy that after genetic testing was found to have two pathogenic variants in arrhythmogenic-related genes after genetic testing.
The index patient was referred to the CardioGenetic's Clinic following a diagnosis of dilated cardiomyopathy detected in a screening echocardiogram for a family history of heart disease. This patient had an uncle with heart transplantation for dilated cardiomyopathy and two other family members with sudden death, an uncle died at 37 yrs old and his son died at 13 yrs old. It was decided to perform genetic testing on the patient with the most severe phenotype, the family member who was submitted to a heart transplant. Previous to the heart transplant, the patient had severe biventricular systolic dysfunction.
A large panel, including genes for cardiomyopathy and arrhythmia, was performed through next-generation sequencing, which revealed a complex genotype: a pathogenic truncating variant in the FLNC gene c.6976C>T, p. (Arg2326)* [NM_001458.4] (ACMG/ACGS 2019:PVS1, PS4_sup, PM2, PP1_str) and also a missense variant at the LMNA gene c.1071C>A, p. (Asp357Glu) [NM _170707.4], not previously reported neither in the literature or populational data; in spite in silico analysis predicted not to be pathogenic, it was in the same residue where other pathogenic variants were reported (ACMG/ACGS 2019:PM1, PM2, PP1_sup, PP2, PM5, BP4). This patient was also found to have two variants of unknown clinical significance, in the DSP gene [NM _004415.3] c.1696G>A, p.(Ala566Thr) and ANK2 gene [NM_001148.4] c.9215A>G p.(Asp3072Gly), possibly benign. Family segregation studies validated the pathogenicity of the LMNA variant, found to be present in two other family members with positive phenotype. Cascade screening revealed additional family member carriers of the truncating FLNC variant in heterozygosity, and carriers of the LMNA variant in heterozygosity. None of other family members were found, yet, to inherited the two pathogenic variants. Two other relatives were already proposed for an implantable cardioverter defibrillator. Sudden cardiac death risk stratification is being performed in all family members available.
This report illustrates the importance of a multidisciplinary team, the absolute benefit of genetic testing in clarifying clinical diagnosis and the importance of awareness for the possibility of unexpected results with utter importance for following up of patients, including primary prevention of sudden death.

## P4 - COMMON MECHANISTIC PATHWAYS IN RARE CONGENITAL SYNDROMES WITH PRIMARY MICROCEPHALY

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Primary microcephaly is an often-seen phenotype in several rare congenital syndromes. It is characterised by a smaller brain size at birth compared to the norm. The causes of this malformation are not fully understood, but genetic testing suggests a connection with defective genes involved in mitotic regulation and proteins related to DNA repair and replication pathways.
Cohesinopathies represent a group of rare syndromes, where several subtypes exhibit spontaneous railroad chromosomes and primary microcephaly. This includes Roberts Syndrome, Warsaw Breakage Syndrome and a recently characterised syndrome caused by mutations in the BUB1 gene. Currently, we are examining fibroblast cells from patients with these syndromes to identify common mechanistic pathways.

In this context, we have identified a new promising candidate: Topoisomerase II alpha, a protein responsible for resolving of the DNA catenation both in the DNA replication and mitosis. Defective localisation of Topoisomerase II alpha may contribute to the observed mitotic defects in these cells. We are currently exploring the impact of these defects on brain development using reprogramming techniques to assess proper neuronal differentiation.

## P5 - TRANSLATIONAL CONTROL OF Δ160P53 KEEPS THE DARK SIDE OF TP53 IN CHECK

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The TP53 tumour suppressor gene was discovered over 40 years ago, but to this day some aspects of its regulation and function remain a mystery. It encodes the full-length p53 protein (FLp53), a transcription factor with a key role in stress response in multicellular organisms, that can either direct cells towards apoptosis or recovery of homeostasis. With such a decisive role, its expression and activity are tightly regulated. A vast set of RNA-binding proteins (RBPs) have been described to affect the translation of FLp53 or the stability of p53 mRNA in response to different perturbations. But in addition to FLp53, there is a group of shorter protein isoforms lacking the N-terminal region, which have well-described functions, and are translated from the same mRNA. The shorter and less studied isoform is Δ160p53, which promotes cell survival, proliferation, and invasion. Despite its usual low levels, it is commonly overexpressed in tumours. However, the detailed mechanisms and factors involved in the regulation of Δ160p53 are still unknown.

In this work, a mass spectrometry was performed to identify the proteins in an RNA-protein co-immunoprecipitation of the p53 mRNA using the MS2 system in the p53-null cell line H1299. The validation of the hits was undertaken by western blot with specific antibodies after immunoprecipitation. The effect of the binding proteins on the translation of Δ160p53 was assessed by overexpression or knockdown, and the expression levels were verified by western blot or luminescence assays.

The mass spectrometry allowed the identification of potential new binding partners of the p53 mRNA. Resorting to the literature and to computational tools available online to predict protein-RNA interactions, a few hits were selected for follow-up and their interactions confirmed. Simultaneously, the modulation of Δ160p53 expression by some of these proteins was verified.

Considering the importance of TP53 in deciding the fate of the cell, the observation of abnormal levels of the oncoprotein Δ160p53 in cancer is intriguing. Understanding the control of its translation could uncover strategies to block it and pave way for new cancer therapies.

## P6 - THE Y-CHROMOSOMAL HAPLOTYPE DIVERSITY OF R1B-M269 SUBHAPLOGROUPS IN INDIVIDUALS FROM THE CENTRAL REGION OF PORTUGAL

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**Introduction:** The most frequent Y-chromosomal haplogroup in the Iberian Peninsula is R1b-M269, representing frequencies of about 60% in Portugal. The R1b-M269 splits into geographically localized subhaplogroups, showing the S116-DF27 branch as the most common in the Iberian Peninsula (40-48%) [1]. We have previously shown that the subhaplogroup DF27 was the most common (70%) among R1b-M269 individuals from Central Portugal [2]. This study aimed to analyze the distribution of the Y-chromosomal haplotypes within the main branches of the R1b-M269 haplogroup in individuals from the central region of Portugal.

**Methodology:** The study sample comprised 54 individuals carrying the derived allele at the M269 SNP from districts of Aveiro, Coimbra, Guarda and Viseu. The internal variation of R1b-M269 samples was assessed by analyzing seven Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393). Multiplex PCR reactions were performed using the QIAGEN Multiplex PCR kit. The forward primers were labeled with Cy5 on the 5' end. The PCR fragments were analysed on an automate ALFexpressTM II sequencer (Amersham Biosciences). The frequencies of the Y-STR haplotypes were determined by direct counting. Population pairwise genetic distances (RSTs) and p-values were calculated using the software Arlequin v3.5.

**Results and Discussion:** The gene diversity of the seven markers ranged from 0.143 (DYS393) to 0.558 (DYS389II) for the sample set. The overall haplotype diversity was 0.343, similar to other Iberian populations (mean 0.368 for five Iberian populations [3]). The total number of observed haplotypes was 31, the most frequent (0.148) was compatible with the "Atlantic Modal Haplotype" (AMH) (DYS19-14/ DYS390-24/ DYS391-11/ DYS392-13/ DYS393-13). The network constructed with the 31 different haplotypes produced a star-like structure with a center occupied by the most common AMH-compatible haplotype (14-13-29-24-11-13-13), shared by eight samples and four different R1b-M269 suclades. The network showed only one missing link, which could indicate evolutionary histories of the R1b-M269 paternal lineages inside the region. The genetic distances between six Iberian populations showed no significant differences, except between Portugal and the Native Basques (RST=0.007; P=0.054).

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## P7 - A 3D CELL CULTURE MODEL OF THE TUBERCULOSIS GRANULOMA THAT CAN BE APPLIED FOR HOST GENETIC STUDIES IN THE CONTEXT OF A MULTICELLULAR IMMUNOLOGIC RESPONSE TO INFECTION

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**Introduction:** The granuloma is an inflammatory infiltrate of mononuclear cells. Some bacterial infections are characterized by the formation of granulomas as part of the immune response to contain the infection. Granuloma models have contributed valuable insights into the genetic basis of granuloma formation during infection. For example, IFNGR1 and IFNGR2 variants have been found to disrupt the immune response, resulting in impaired granuloma formation and increased susceptibility to diseases by Mycobacterium sp. More easily implemented comprehensive models would facilitate the study of the different immune mechanisms and help identify new disease-associated genes. Our objective is to generate an in vitro 3D cell culture model using human primary cells and microspheres to generate a stratified granuloma model for future use in genetic, immunological and drug discovery studies.

**Methods:** A commercial system was used to encapsulate human peripheral blood mononuclear cells (PBMC) infected with GFP-expressing M. tuberculosis and maintained in culture for several weeks. The cellular constituents of these granulomas and their organization were characterized by fluorescence microscopy and flow cytometry as well as the viability of the cells and the extent of bacterial replication in factor of time.

**Results:** The results demonstrate a ready recruitment of cells towards infected macrophages, leading to the formation of densely populated aggregates. These aggregates maintained cell viability for several weeks and displayed an enhanced control of bacterial replication compared to the more common monolayer infection models. Moreover, the capsules can be easily disrupted when required to isolate genetic material for further analysis.

**Conclusion:** The proposed 3D model resembles some structural and cellular characteristics of the tuberculosis granuloma and maintains its stability beyond more common 2D models of infection. These preliminary results demonstrate that this model can be used to further explore the determinants of granuloma formation and host response to infection.

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