Group **A** Poster n. **1**

Section n. 1. Concept of species in the genomic era: past, present and future

Environmental DNA detects genetic diversity and ecological features of eukaryotic species communities in Mediterranean transitional waters

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Climate changes and anthropogenic pressures are the main drivers of a biodiversity decline characterized by an unprecedented rate of species extinction. In addition to a decrease in species composition, habitat reduction and degradation are also causing a loss of species' genetic diversity. The genetic diversity of natural populations and communities ensure their adaptive potential and evolvability. We applied environmental DNA (eDNA) metabarcoding to investigate the biodiversity of a poorly known Mediterranean lagoon included in the European Natura 2000 Network. We used the cytochrome oxidase I (COI) and ribosomal rRNA 18S as marker genes to explore the eukaryotic biodiversity of this aquatic coastal environment. This high throughput molecular surveying unveiled a wide variety of taxonomic groups and provide insights into genetic diversity of eukaryotic species of these transitional waters. The results demonstrated the validity of eDNA studies to establish the genetic and ecological structure, as well as the spatial variation in response to environmental variables, of eukaryotic communities in transitional aquatic ecosystems. This study outlines the importance of genetic diversity surveying for conservation strategies of natural ecosystems.

Group **A** Poster n. **2**

Section n. 2. Environment and epigenetic variation

Natural compounds modulate chromatin plasticity in in-vitro Preeclampsia model

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Preeclampsia (PE) affects approximately 2-8% of pregnant women and is the leading cause of maternal and perinatal mortality. Currently, the treatment of PE includes rest and blood pressure control. Therefore, we decided to characterise PE using placenta biopsies from affected women and controls, highlighting a specific inflammatory and chromatin pattern. After that, we focused on generating an in vitro model of PE to test the cytotoxicity of 4 bio-compounds, evaluate the protective role of natural substances against inflammation, and study the epigenetic modifications at the level of the global chromatin state. The human trophoblast HTR8-SVneo, treated with TNF-α, was used as inflammatory model of placenta. To test the protective role of natural compounds on PE model, we analysed the cell vitality after pre-treatment with the compounds for 24h, followed by TNF-α inflammation. We have shown a significant accumulation of IKB-α protein and a down-regulation of IL-8 and COX-2 gene expression in the cells pre-treated with natural compounds. Finally, we evaluated the global epigenetic remodelling by histone modifications protein. Our data showed for the first time that pre-treatment with bio-compounds increased H3k4me2 protein level and decreased H3k9me3; the trend reversed in cells treated with TNF-α alone. This preliminary study allowed us to generate an in-vitro PE model and verify the efficiency of some bio-compounds in the modulation of inflammatory targets and histone proteins.

Group **A** Poster n. **3**

Section n. 2. Environment and epigenetic variation

Multilevel interactions of drought signals with the floral genes network

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Plants deploy several strategies to endure remarkable levels of drought stress, including the synchronization of flowering with the rainy season to anticipate summer drought. This strategy, referred to as drought escape (DE) can also be plastically adjusted in Arabidopsis. Two signalling networks form the basic structure of DE; the photoperiodic pathway, and the phytohormone abscisic acid (ABA). Photoperiodic genes and ABA converge upon the transcriptional activation of the florigen gene FT. Florigen activation is mediated by CONSTANS (CO) and the study of transgenic plants over-expressing tagged versions of CO proteins in different ABA genetic backgrounds points to a post-transcriptional level of regulation of CO mediated by ABA. ABA does not alter diel CO protein accumulation, but rather its recruitment to the FT proximal promoter. The diminished recruitment of CO in ABA-deficient mutants impairs RNApol2 occupancy around the transcription start site of FT and the initiation of its transcription. Another photoperiodic protein, GIGANTEA (GI) participates in this process, although its precise function requires further investigations. Interestingly, we found a large genetic variation for the DE trait, including populations of Arabidopsis showing much reduced DE response compared with the reference Col-0 ecotype. Our data suggest that plants may utilize ABA signals in different ways to coordinate flowering time according to the prevailing watering conditions to maximise fitness.

Group **A** Poster n. **4**

Section n. 2. Environment and epigenetic variation

The temporal profile of circulating microRNA induced by acute experimental pain

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In the last years, microRNA (miRNA) emerged as important regulators of pain development. The temporal expression of circulating miRNA was investigated in response to an acute, experimental, pain provocation realized through intramuscular hypertonic saline injection in the plasma of healthy volunteers. Twenty volunteers were randomly allocated into two groups: the pain group receiving a hypertonic saline injection and the control group receiving an isotonic saline injection in the first dorsal interosseous muscle of their dominant hand. Pain intensity was continuously recorded for 20 minutes after injection using the VAS scale. MiRNA were extracted from plasma of blood samples taken at baseline, 30 minutes, 3 hours, and 24 hours post-injection. RNA sequencing with the Illumina NextSeq platform was performed at each time point comparing miRNA expression between pain and control groups. Significant differences were considered when folds were >2 and the False Discovery Rate was $p < 0.05$. Differentially expressed miRNA were identified comparing pain and control groups (4 miRNA after 30 minutes, 24 miRNA after 3 hours and 42 miRNA after 24 hours from baseline, $p < 0.0001$). Two miRNA were consistently upregulated throughout the experiment. The identified miRNA are involved in brain perception of pain, brain signaling and response to stimuli. These fast modifications in gene expression induced by acute experimental pain should be further investigated.

Group **A** Poster n. **5**

Section n. 2. Environment and epigenetic variation

Evaluation of epigenetic aberrations in workers exposed to titanium dioxide (TiO2): analysis of an early DNA methylation alteration associated with cancer development

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The development of human diseases is influenced by genetic and environmental factors. The interplay between these factors is mediated by epigenetic changes. One of the main focuses of epigenetic research is the study of cancer, that traditionally was viewed as a genetic disease but it is now clear that its onset is preceded by epigenetic abnormalities while the genetic modifications occur later. The study of epigenetic alterations has also a particular relevance in understanding the effect of occupational exposures to pollutants and new materials, such as titanium dioxide (TiO2) nanoparticles and nanofibers, which are promising candidates for numerous applications in consumer products, but with potential toxic effects.
The aim of this project is to investigate whether TiO2 represents a risk to human health by analysing a proprietary early colorectal cancer biomarker using MethyLight on plasma samples of 45 subjects exposed to TiO2, compared to 18 external controls and 19 cancer patients. Preliminary results show high methylation levels in cancer patients, low or negligible in controls and intermediate in exposed subjects, with values very similar to those of cancer patients for some categories of workers. More in-depth multivariate analyses, taking into account possible confounding factors, will be conducted, as well as extending the sample series. These results, although preliminary, seem to confirm a potential risk conferred by TiO2 exposure on human health.

Group **A** Poster n. **6**

Section n. 2. Environment and epigenetic variation

Histone Deacetylase Complex 1 and Histone 1 epigenetically moderate stress responsiveness of Arabidopsis thaliana seedlings

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Early responses of plants to environmental stress factors prevent damage but can delay growth and development in fluctuating conditions. Optimising these trade-offs requires tunability of plant responsiveness to environmental signals.
We have previously reported that Histone Deacetylase Complex 1 (HDC1), which interacts with multiple proteins in histone deacetylation complexes, regulates stress-responsiveness of Arabidopsis seedlings, but the underlying mechanism remained elusive.
Here, we show that HDC1 attenuates transcriptome re-programming in salt-treated seedlings and we identify two genes (LEA, MAF5) that inhibit seedling establishment under salt stress downstream of HDC1. HDC1 attenuates their transcriptional induction by salt via a dual mechanism involving H3K9/14 de-acetylation and H3K27 trimethylation. The latter, but not the former, was also abolished in a triple knockout mutant of the linker histone H1, which partially mimic hyper-sensitivity of the hdc1-1 mutant to salt stress. Although stress-induced H3K27me3 accumulation required both H1 and HDC1, it was not fully recovered by complementing hdc1-1 with a truncated, H1-binding competent HDC1 suggesting other players or independent inputs.
The combined findings reveal a dual-brake function of HDC1 via regulating both active and repressive epigenetic marks on stress-inducible genes. This natural ‘anti-panic’ device offers a molecular leaver to tune stress responsiveness in plants.

Group **A** Poster n. **7**

Section n. 2. Environment and epigenetic variation

Tracing the dynamics of ADAR-mediated RNA editing during Ostreid herpesvirus-1 infection

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Enzymes of the adenosine deaminase acting on dsRNA (ADAR) family can perform post-transcriptional modifications on structured RNAs, by converting Adenosine in Inosine (A-to-I editing), eventually diversifying transcriptomes and then proteomes. Extensive ADAR-mediated editing of Ostreid herpesvirus-1 (OsHV-1) RNAs occurs during infection of the Pacific oyster Crassostrea gigas as a part of an interferon-like response. Here, we investigated the transcriptional responses of the blood clam Scapharca (Anadara) broughtonii along a 72-hours' time-course infection experiment with OsHV-1, to reveal the extent of RNA editing and its impact on host and viral genomes. Illumina sequencing was used to trace transcriptional changes and detect ADAR hyper-editing events, whereas long-read RNA sequencing was used to investigate transcriptional architectures.
ADAR editing increased along the viral infection, with a preferential impact on OsHV-1 RNAs. ADAR-mediated edits were concentrated in a few hotspots, corresponding to overlapping antisense gene regions, which were increasingly produced during infection by 5'-read-through transcription. We also reported a significant underrepresentation of the dinucleotide along the OsHV-1 genome, representing preferential ADAR targets.
Overall, we suggested that ADAR activity has been lowered and concentrated on dedicated molecular decoys, likely due to virus-host co-evolutionary mechanisms.

Group **A** Poster n. **8**

Section n. 2. Environment and epigenetic variation

TET1 and TDG suppress inflammatory response in intestinal tumorigenesis: implications for colorectal tumors with the CpG Island Methylator Phenotype

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Background/Aim: Aberrant DNA methylation is common in colorectal cancer (CRC), but underlying mechanisms and pathological consequences are largely unknown.
Methods: We explored the role of active DNA demethylation in intestinal tumorigenesis, by inactivating Tet1 and/or Tdg in ApcMin mice and characterizing the methylome and transcriptome of colonic adenomas. Data were compared to TCGA human colonic adenocarcinomas (COAD).
Results: Tdg-mutant ApcMin mice developed an increased number of small intestinal adenomas, whereas Tet1-mutant and Tet1/Tdg-double heterozygous ApcMin mice showed larger colonic adenomas with invasion features. We detected reduction in global DNA hypomethylation in colonic adenomas from Tet1- and Tdg-mutant ApcMin mice, and hypermethylation of CpG islands in Tet1-mutant ApcMin adenomas, which resembles the CpG island methylator phenotype (CIMP) in human CRC. Increased inflammatory/immune responses were present in Tet1- and Tdg-mutant colonic adenomas compared to control ApcMin adenomas. An inflammatory signature split COAD into 4 groups, linked to their genomic instability, and characterized by different levels of DNA methylation, DNMT1 and TET1 expression. CIMP tumors had concerted high DNMT1/low TET1 expression.

Conclusions: We reveal a new epigenetic regulation by which TET1-TDG-mediated DNA demethylation decreases methylation and inflammatory/immune responses. CIMP in CRC is caused by an imbalance of methylating activities over demethylating activities.

Group **A** Poster n. **9**

Section n. 2. Environment and epigenetic variation

DNA METHYLATION ALTERATIONS IN LEISHMANIA-INFECTED MACROPHAGES AND UPON DRUG TREATMENT

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Leishmaniasis, a neglected tropical disease caused by the protozoan Leishmania, presents different clinical forms, ranging from cutaneous/mucosal lesions to visceral infections. The flagellated promastigote stage of Leishmania enters in macrophages and transform into amastigote. The parasite colonizes macrophages by modulating host cells epigenome, including DNA methylation. Given the lack of effective vaccines and therapy toxicity, leishmaniasis represents a public health problem. The study of DNA methylation alterations due to Leishmania species could lead to a new research area for leishmaniasis therapy. Therefore, we performed a genome-wide methylation analysis in macrophages, in presence/absence of IL6, infected with L. braziliensis, L. amazonensis and L. infantum. Different DNA methylation alterations were identified upon infection and among species, suggesting a specie-specific parasite/host interaction. Moreover, macrophages infected with L. braziliensis and L. infantum were treated with Glucantime and Amphotericin B. The differential methylation analysis performed between treated and untreated samples showed distinct DNA methylation profiles. We detected a tendency to the reestablishment of the DNA methylation towards a normal pattern after treatment. The discovery of DNA methylation alterations among species and upon treatment addresses further functional studies and provides a probable new approach for leishmaniasis therapy.

Group **A** Poster n. **10**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

BET inhibitors rewire metabolism in the aged skeletal muscle

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Aging is associated with a progressive decline of muscle mass and strength, that is observed among healthy adults, with an acceleration in the rate of decline past middle age. The pathological loss of muscle mass associated with aging, known as sarcopenia, negatively affects the quality of life and leads to increased occurrence of falls, hospitalization, and to decreased independence. Previous reports from our group showed that the bromodomain and extra-terminal domain (BET) protein BRD4 plays a role in promoting muscle wasting in experimental models of cancer cachexia and muscular dystrophy. Here, we evaluated the impact of pharmacological blockade of BET proteins in the skeletal muscle of 24-month-old mice.

Mice were treated with the BET inhibitor JQ1+ (20mg/kg) or the inactive enantiomer JQ1- daily, for 24 days. During treatment, mice were weighed, and muscle performance was evaluated through the treadmill and grip tests. After sacrifice, different muscles and several tissues were isolated and collected for morphological and molecular analysis, including RNA-seq, Western Blot, and IHC.

Our data show that JQ1 treatment induced weight loss in old mice and BET blockade also displayed a beneficial effect on muscle performance, and it was associated with a marked reduction in fibrosis. Following JQ1 treatment, RNA-seq assays highlighted an enrichment in the level of key transcripts involved in fatty acid oxidation in skeletal muscle. Metabolomic and immunoblot analysis revealed a reduced reliance on glycolysis and an increase in fatty acid oxidation. In conclusion, our data suggest that JQ1+ treatment ameliorates mitochondrial fatty acid metabolism in old mice, improves muscle function and it may be beneficial in the treatment of sarcopenia.

Group **A** Poster n. **11**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

Exploiting yeast for functional characterization of novel missense DARS2 variants identified in siblings presenting leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation

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Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is a neurometabolic disorder caused by mutations in the nuclear gene DARS2, encoding the mitochondrial aspartyl-tRNA synthetase. Yeast has been widely exploited to determine the pathogenicity of mutations identified in patients, including those that occur in genes encoding mitochondrial tRNA synthetases. The yeast homolog of human DARS2, MSD1, encodes the mitochondrial isoform of aspartyl-tRNA synthetase. We used yeast as a model to study the effect of two novel DARS2 variants identified in compound heterozygosis (p.E158V and p.E277K) in two siblings. To study the pathogenicity of human mutant alleles the corresponding yeast strains were created (msd1Q137V and msd1E259K), with the additional humanized strain (msd1hQ137E) due to the non-conservation of human p.E158 residue. Strains' oxidative growth ability and respiratory activity were assessed to evaluate the impact of the variants on mitochondrial function; the strain msd1E259K showed a severe defect, whereas msd1Q137V showed a leaky defect. Overall, the results indicated that both variants are deleterious in yeast thus validating the two human variants as pathogenic; in particular, DARS2 E277K is an amorphic allele, whereas E158V is a hypomorphic allele. In conclusion, this study highlights the utility of the yeast model as a functional tool to study variants in genes related to mitochondrial disorders.

Group **A** Poster n. **12**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

High-fat diet and susceptibility to metabolic syndrome: beyond the genotoxic role of oxidative DNA lesions

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Body fat mass increase is linked to oxidative stress and the accumulation of radical oxygen species (ROS) contributes to develop the metabolic syndrome. ROS induce DNA damage with a consequent activation of DNA damage response, which, if permanently activated, is able to trigger systemic inflammation.
Mice defective in DNA repair genes are highly susceptible to upon high-fat diet (HFD), suggesting a link between genomic instability and metabolic dysfunction.
Our mouse model (hMTH1-Tg) overexpresses hMTH1, a hydrolase which protects cells by oxidative damage. When compared with wild-type (wt), hMTH1-Tg shows: decrease in oxidative DNA damage; increased longevity; delay in the ageing process; best mitochondrial functionality; protection against neurodegeneration; a better cognitive-behavior profile.
In this study, we show that MTH1 modulates oxidative DNA damage HFD-induced as well as during aging. Moreover, up to 4-weeks of HFD, hMTH1-Tg is characterized by a higher content of brown adipose tissue and lower liver fat accumulation. Surprisingly, chronic exposure to HFD leads to loss of the hMTH1-Tg advantageous phenotype. Interestingly, the return to normal diet was able to restore basal levels of oxidative DNA damage as well as cholesterol and alanine aminotransferase. Altogether, our results show that oxidative DNA damage is directly linked to diet and support evidence of an epigenetic role of oxidative DNA lesions in energy homeostasis.

Group **A** Poster n. **13**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

Exploiting yeast for the validation and characterization of COQ7 variants and identification of novel potential treatments for primary CoQ10 deficiency

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Primary CoQ10 deficiency is a group of inborn errors of metabolism determined by defects in CoQ10 biosynthesis, including mutations in COQ7. Yeast S. cerevisiae, a widely used model organism to study mitochondrial diseases, has been exploited to validate and characterize newly identified and previously reported mutations thanks to the presence of the ortholog gene CAT5, by creating the corresponding yeast strains. All variants have been validated and can be divided into severe mutations or leaky mutations based on the oxidative growth defect and the residual respiratory activity of the yeast models. Despite some attempts to use CoQ10 supplementation, no effective treatment for primary CoQ10 deficiency exists. Supplementation of 2,4 dihydroxybenzoic acid (2,4-DHB) together with CoQ10-biosynthesis complex stabilization through COQ8 overexpression, was previously shown to rescue the oxidative growth defect of cat5Δ yeast strain. We demonstrated that supplementation with 2,4-dHB, with or without COQ8 overexpression, rescues the mitochondrial defect of both the leaky and severe mutants. In addition, the “combo” treatment determines a significant improvement of respiratory complexes (RC) subunits levels on strains carrying cat5Y154C and cat5V146E variants, characterized by a reduction in the RCs . Overall, the results expand the phenotypic spectrum of COQ7-associated primary CoQ10 deficiency and lead to the identification of a novel potential treatment for primary CoQ10 deficiency.

Group **A** Poster n. **14**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

Mitochondrial dynamics analysis during ER stress induced by dyskerin downregulation

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Dyskerin is a key component of both telomerase and snoRNA H/ACA complex, which acts as a pseudouridine synthase, guiding modifications mainly in ribosomal RNA and snRNA, and whose malfunction cause the X-linked dyskeratosis congenita disorder. In a previous study, we showed that dyskerin depletion induces the accumulation of unfolded/misfolded proteins in the endoplasmic reticulum (ER), which in turn causes the PERK branch activation of the unfolded protein response (UPR). ER stress can be transmitted to the mitochondria through Mitochondrial Associated Membranes (MAMs) and determine an erroneous function of the organelle that could lead to different pathologies. In this study, we analyzed the effects of ER stress, and the consequent activation of UPR, caused by dyskerin depletion, at mitochondrial level. In particular, we analyzed some key mitochondrial fusion markers, which could be involved in replacement of aged or damaged mitochondria. We report that in dyskerin-depleted cells ER stress induces an increase of mitochondrial fusion without activation of mitophagy, indicating that this process could protect cells from ER stress insults. These findings suggest that dyskeratosis congenita patients could benefit from drugs already clinically approved for use in other pathologies, like Parkinson's disease, in which mitophagy is not correctly activated. In conclusion, this could represent a new factor to be considered in the search for a possible cure for dyskeratosis congenita.

Group **A** Poster n. **15**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

DNA methylation levels of the mitochondrial D-loop region correlate with amyotrophic lateral sclerosis disease duration

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Mitochondrial epigenetic mechanisms are emerging as important players in the pathogenesis of several human diseases, including neurodegenerative ones. We have previously reported altered methylation patterns of the mitochondrial D-loop region, which regulate mitochondrial DNA (mtDNA) replication, in the peripheral blood of Amyotrophic Lateral Sclerosis (ALS) patients with different genetic etiology.
In the current study, we searched for correlations between D-loop methylation levels and mtDNA copy number with disease duration in familial ALS patients.
Peripheral blood was collected from 12 patients with pathogenic variants in SOD1 gene and 13 patients with the C9orf72 hexanucleotide repeat expansion. Pyrosequencing and quantitative real-time PCR were used to evaluate D-loop methylation levels and mtDNA copy number, respectively.
D-loop methylation levels were not affected by the sex and age of the patients, were lower in SOD1-ALS when compared to C9orf72-ALS patients ($p < 0.0001$), and were strongly inversely correlated with the duration of the disease ($r = -0.67$; $p = 0.0002$). On the other hand, mtDNA copy number positively correlated with disease duration ($r = 0.38$; $p = 0.056$).
Given the central role played by mitochondrial dysfunction in ALS, this study provides new advances for future investigations aimed at identifying epigenetic biomarkers sensitive to the progression of the disease as well as new biological targets for therapeutic actions.

Group **A** Poster n. **16**

Section n. 3. Mitochondrial (dys)function in non-communicable diseases

Assessing an unprecedented role for Heterochromatin Protein 1a (HP1a) at mitochondria

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The Drosophila Heterochromatin Protein 1a (HP1a) is a critical and conserved non-histone protein with essential roles in heterochromatin formation/maintenance and heterochromatin-related gene silencing. We recently found that HP1a is also present in the mitochondria fraction, mostly associated with the outer mitochondrial membrane and with Drosophila VDAC1 indicating that it could be considered a mitochondria resident protein. Cellular fractionation from human cells and from mouse liver and heart samples indicated that also the mammal ortholog Hp1a localized at the mitochondria, suggesting that HP1a plays evolutionarily conserved roles in this organelle. Interestingly loss of HP1a in Drosophila and in human cells yielded increased mitochondria number and fragmentation as well as an accumulation of mature autophagosomes. Moreover, HP1a depleted human cell lines exhibited high levels of the two mitophagy receptors BNIP3 and NIX suggesting that HP1a plays a role in the regulation of Mitochondrial Quality Control. Finally, a specific depletion of HP1a in Drosophila larval and adult muscles impaired locomotor activity and affected mitochondria ultrastructure, indicating that an HP1-dependent mitochondria regulation is required at multiple levels during development.

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Section n. 7. General genetics and genomics

The nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG) sensitize breast cancer cells to ionizing radiation exposure

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The nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG), currently in clinical trial, has been shown to inhibit the proliferation of telomerase-positive cancer cells through its incorporation into telomeres and subsequent induction of DNA damage, cell cycle arrest and cell death. Since telomere targeting has been proposed as a promising strategy to increase sensitivity of tumour cells to ionizing radiation, in the present work we aimed to test the efficacy of 6-thio-dG and X-rays combined treatments in breast cancer cell lines.
In order to identify the best conditions for combined treatments, the anti-proliferative effect, the cell cycle modulation and the telomeric DNA damage induction were assessed both in telomerase-positive breast cancer lines (MCF7, HACC-1937, MDA-231) and in human primary fibroblasts, used as controls.
Based on these results, combined treatment experiments were performed. Surviving fraction experiments indicated that 6-thio-dG is able to increase the radiosensitivity in breast cancer MCF7 cells, interacting in synergistic manner with X-rays. Moreover, data has been confirmed also on a three-dimensional model, by using microencapsulation of MCF7 cells in alginate hydrogels, through the live/dead staining-cell viability assay. In addition, apoptosis and molecular cytogenetic analysis were carried out to support results obtained.
Data so far collected confirm telomere targeting as a promising radiosensitizing strategy in breast cancer cells.

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Section n. 7. General genetics and genomics

Mutants alleles in Aicardi-Goutières Syndrome patients produce a disfunctional RNASET2 protein associated with alterations in interferon signalling.

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Aicardi-Goutières Syndrome (AGS), a genetically determined, early onset Mendelian encephalopathy displaying phenotypic overlap with systemic lupus erythematosus and viral congenital infections, has been linked to mutations in several human genes involved in nucleic acid sensing, signaling or processing. A typical feature of AGS includes an "interferon signature", represented by increased levels of both interferon-alpha (IFN-□) and IFN-□-regulated genes in cerebrospinal fluid and peripheral blood, which collectively define AGS as an inflammatory disease triggered by induction of an excessive type-I interferon-mediated innate immune response, possibly driven by the accumulation of endogenous nucleic acids.
The RNASET2 gene encodes the only human member of the highly conserved T2/Rh/S family of extracellular ribonucleases and has been consistently reported to be involved in innate immune response regulation.
Here, we show that several mutant RNASET2 alleles carried by AGS patients encode a functionally defective protein, whose catalytic activity, intracellular distribution, and secretion pattern are significantly impaired, compared to the wild-type allele-encoded protein. Furthermore, expression of the mutant alleles in the RNASET2-null MCF7 cancer cell line was found to be associated with the AGS-associated interferon signature.
We thus propose human RNASET2 as novel member of the increasing family of nucleic acid sensing/signalling genes involved in the pathogenesis of AGS.

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Section n. 7. General genetics and genomics

Accumulation of 8-oxodG within human mitochondrial genome positively associates with transcription.

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Mitochondrial DNA (mtDNA) can be subject to internal and environmental stressors that lead to oxidatively generated damage and the formation of 8-oxo-7,8-dihydro-2'-deoxyguanine (8-oxodG). The accumulation of 8-oxodG has been linked to degenerative diseases and aging, as well as cancer. Despite the well-described implications of 8-oxodG in mtDNA for mitochondrial function, there has been no reports of mapping of 8-oxodG across the mitochondrial genome. To address this, we used OxiDIP-Seq and mapped 8-oxodG levels in the mitochondrial genome of human MCF10A cells. Our findings indicated that, under steady-state conditions, 8-oxodG is non-uniformly distributed along the mitochondrial genome, and that the longer noncoding region appeared to be more protected from 8-oxodG accumulation compared to the coding region. However, when the cells have been exposed to oxidative stress 8-oxodG preferentially accumulated in the coding region which is highly transcribed as H1 transcript. Our data suggest that 8-oxodG accumulation in the mitochondrial genome is positively associated with mitochondrial transcription.

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Section n. 7. General genetics and genomics

DNA Damage induced by Ionizing radiation activates the innate immune response: study and characterization of the pathway cGAS-STING.

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Ionizing radiation (IR) has been shown to modulate immune response processes. IR-mediated immune system modulation involves several players (such as stress sensors and cytokines) regulating immune and inflammatory response. Among them, the cyclic synthase-GMP-AMP (cGAS), a free cytosolic DNA sensor, is capable of recognizing DNA fragments that, after treatment with IR, manifest as micronuclei (MNi)1. Activation of the cGAS causes GAMP dependent-STING translocation from ER to ER-Golgi. STING activation promotes phosphorylation and translocation into the nucleus of transcription factors (IRF3 and IRF7) that induce innate immune response and type I interferon (IFN)2. In the present work the induction of cGAS-positive MNi was evaluated in immortalized human keratinocytes (HaCaT) exposed to X-rays (250 Kev; 0.5, 1 and 2Gy) and fixed 24-120h after treatment, to determine the dose-response and activation kinetic. In addition, type I IFN gene expression, such as IFN alfa and beta, and ISG15 protein levels were analyzed by RT-qPCR and western blotting. Other experiments are in progress to understand whether the nuclear lamina integrity has a role in the exposure of DNA in the cytoplasm with consequent recognition by cGAS. Confirm the role of DNA damage in immune system activation will provide useful information for studies on radioimmunotherapy.

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Section n. 7. General genetics and genomics

Caspase-8: an unexpected role in glioblastoma progression and resistance to radiotherapy

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Glioblastoma (GBM) is the most frequent and lethal malignant primary adult brain tumor, which is characterized by poor prognosis due to chemo- and radio-therapy resistance and tumor recurrence. Therapy resistance is mainly caused by intra and inter tumor heterogeneity and by neoangiogenesis, and by the aberrant functionality of DNA repair mechanisms, which represent two of the most important hallmarks of GBMs.
Caspase-8 is a cysteine protease promoting apoptosis, whose expression is unexpectedly often retained in some tumors, including GBM, suggesting the presence of alternative pro-tumoral functions. In this regard, we reported that the. aberrant activation of Src kinase can in cancer can trigger Caspase-8 phosphorylation on Tyrosine 380 (Y380) which prevents Caspase-8 full activation and thereby inhibits apoptosis. By investigating the significance of Caspase-8 expression and of its phosphorylation on Y380 in GBM, where both Caspase-8 expression and Src activity are often aberrantly upregulated, we identified a new role of Caspase-8 expression and phosphorylation as promoter of the expression of inflammatory and pro-angiogenetic factors, sustaining neoangiogenesis and resistance to radiotherapy. In addition, we provide evidence for Caspase-8 as a new player in DNA repair functionality in response to ionizing radiation. In summary, our work identifies a novel role of Caspase-8 as modulator of tumor progression and resistance to therapy.

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Section n. 7. General genetics and genomics

Unmasking LQT1 syndrome phenotype by maturing hiPSC-cardiomyocytes in cardiac microtissues

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The KCNQ1 gene encodes the cardiac channel conducting the slow component of the inward repolarizing potassium current (IKs) and loss-of-function mutations cause the life-threatening arrhythmia type 1 long-QT syndrome (LQT1). KCNQ1 is paternally imprinted in most adult tissues, but in the heart imprinting is lost during development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely used to model LQT1, however their typical immaturity may affect imprinting in KCNQ1.
We compared hiPSC-CMs from a LQT1 patient carrying a KCNQ1 mutation on the paternal allele with the isogenic corrected line. We evaluated KCNQ1 allelic expression and functional properties in hiPSC-CMs both in standard 2-dimensional (2D) culture and after maturation in 3D cardiac microtissues.
No difference in action potential duration (APD) was detected between LQT1 and corrected hiPSC-CMs, suggesting that imprinting was masking LQT1 phenotype. Indeed, 2D hiPSC-CMs exclusively expressed one KCNQ1 allele. By maturing hiPSC-CMs in cardiac microtissues (MTs), the paternal imprinting was partially lost, thus revealing APD prolongation.
This study brings attention to hiPSC-CM epigenetic regulation, showing that residual KCNQ1 imprinting can lead to underestimation of functional effects of pathological variants. This is overcome by maturing hiPSC-CMs in MTs, revealing the LQT1 phenotype; this approach represents a more reliable in vitro preclinical model.

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Section n. 7. General genetics and genomics

BLOOD BACTERIAL DNA IN THE MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is a chronic and autoimmune demyelinating disease of the central nervous system that affects approximately 2.5 million people and it is more prevalent in females than in males. The main characteristic of the disease is the development of inflammatory plaques, areas of focal demyelination present in both white and grey matter of the brain and spinal cord. In the last years, numerous studies have reported the presence in the bloodstream of bacterial DNA (BB-DNA) in both physiological and pathological conditions, whose origins and functions are still to be clarified.
The present study aimed to investigate the presence and, then, the levels of BB-DNA by quantitative Real-Time PCRs targeting the 16S rRNA genes in blood from subjects affected by MS. The results obtained showed significantly higher levels of BB-DNA in affected compared to healthy subjects, thus, providing, for the first time, evidence about the association of BB-DNA with MS. Interestingly, in the affected, these levels correlate positively with smoking habits, confirming that lifestyles and environmental factors are significant contributors. Further studies should be performed to understand whether the BB-DNA might play a causal role or indirect effect in MS pathophysiology and elucidate whether such DNA can become an effective prognostic marker of the onset and/or disease progression.

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Section n. 7. General genetics and genomics

Deciphering the transcriptional network of NF-Y in prostate cancer development and progression.

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The transcription factor NF-Y has a demonstrated role in cancer metabolism and cooperates with SREBPs, the master transcription factors of cholesterol and lipid production. In prostate cancer (PCa), androgens signalling can modulate SREBPs and rewire lipid metabolism.
We demonstrated that high NF-YA expression and an increased ratio between the two NF-YA alternatively spliced transcripts (NF-YAs/NF-YAl) characterize PCa tissues. NF-YA loss reduces tumor development, while NF-YAs or NF-YAl overexpression increases tumor growth or cell motility, respectively.
According to our findings, NF-Y directly regulates Androgen Receptor (AR) expression and affects Androgen deprivation therapy (ADT) and AR-inhibitors (ARSI) sensitivity. The bioenergetic metabolic profiling confirmed the key role of NF-Y in fatty acid metabolism in LNCaP PCa cells.
In normal epithelial prostate RWPE1 cells, NF-YAl overexpression decreased cell migration and increased clonogenic growth. RNA-seq profilings showed the existence of different NF-YAl and NF-YAs-associated regulomes in prostate cells. Cholesterol homeostasis and SREBP-dependent gene expression are the top-deregulated pathways in NF-YAl-overexpressing cells, while extracellular matrix organization and EMT are affected by NF-YAs overexpression.
Overall, the characterization of NF-Y transcriptional pathways is important to understand prostate pato-physiology and may help the stratification of PCa patients to predict ADT/ARSI sensitivity.

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Section n. 7. General genetics and genomics

A novel role of the RTEL1 helicase in cell cycle progression and sister chromatid cohesion

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RTEL1 belongs to the iron-sulfur cluster (Fe-S) family of helicases and is well-known in scientific literature for its involvement in maintaining telomeric stability. Similar to other helicases within this family (such as FANCJ, BLM, DDX11, and XPD), RTEL1 plays a crucial role in resolving secondary DNA structures and facilitating the replication of telomeric regions. Despite its structural similarity to the DDX11 helicase, which is associated with cell cycle progression and sister chromatid cohesion, there is no evidence in the literature suggesting a comparable function for RTEL1.
To explore whether RTEL1 served any extratelomeric roles, we conducted gene silencing experiments in a panel of tumor cell lines. Data from these experiments indicate that RTEL1 gene silencing leads to an accumulation of cells in mitosis and/or to mitotic slippage, resulting in the formation of polyploid cells. This blockage during the M-phase occurs independently of the p53 protein but relies on premature chromosome separation (PCS) phenomena, which were significantly higher in the silenced cells.
These findings have unveiled a novel role for RTEL1, distinct from its telomeric function, in promoting sister chromatid cohesion and facilitating mitotic progression. This discovery paves the way for future studies aiming to characterize the molecular mechanism behind this role and its involvement in the activation of the mitotic checkpoint.

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Section n. 7. General genetics and genomics

The genetic structure of sweet chestnut trees varieties in the Lario region

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Conservation genetics can, and must, be an important asset in planning conservation strategies that involve a direct engagement of the local people. The presented work is a first attempt at mapping the different varieties of sweet chestnut (Castanea sativa L.) of the Eastern Lario region in Lombardy. In this area the cultivation of chestnut trees dates back to the Roman period and is now characterized by an extremely high number of "local" varieties, each with its own name and localization. We have started genotyping ten of the most diffused varieties by means of SSRs, with the ultimate goal of checking whether the attribution of the plants to historical entities could be confirmed by genetic analysis. Concurrently, a morphological characterization of the trees is under way. Genetic diversity, genetic differentiation and genetic structure have been assessed, showing that the actual state is a mixed one: several historical varieties do represent genetic entities, while others indicate a more complex relationship. The demographic history was also studied through a coalescent-based approximate Bayesian computation. The results will allow the recovery and conservation of the native genetic heritage at risk of erosion and abandonment and to select varieties able to respond better to environmental changes; this will also be obtained by the implementation of a "common garden" localized within the studied area, so as to involve the local people in the protection of biodiversity.

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Section n. 7. General genetics and genomics

The role of BLM and FANCJ DNA-helicases in the response to G-quadruplex-stabilizing ligand RHPS4

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G-quadruplexes (G4s) are secondary DNA structures that arise spontaneously in guanine-rich regions (e.g. telomeres). G4s have a role in regulating important biological functions but they might represent an obstacle for DNA metabolism.
To prevent G4-related genomic instability, their unwinding is mediated by several proteins such as BLM and FANCJ helicases. Data from literature indicate that FANCJ physically and functionally interacts with BLM, collaborating in the cellular response to replicative stress (RS).
In this study we focused on the role of BLM and FANCJ in response to a well-known G4 stabilizer compound, RHPS4, able to induce telomeric RS. To this end, we generated CRISPR/Cas9 U251MG glioblastoma cell lines knock-out (KO) for BLM and silenced for FANCJ, in order to evaluate single and combined contribution of the two helicases in response to RHPS4 treatment.
Whereas BLM depletion did not affect cell proliferation, siFANCJ cells displayed a moderate cell growth delay and defects in telomeric replication upon RHPS4 treatment. In BLM/FANCJ depleted cells, proliferation was totally inhibited already in the absence of G4-ligand treatment and this evidence correlates with a significant increase in telomeric defects and chromosomal aberrations. Further experiments are in progress to clarify the mechanisms involved in RS response to telomeres and assess whether G4-ligands may be an attractive therapeutic strategy to overcome chemo-resistance in cancer cells.

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Section n. 7. General genetics and genomics

Disentangling the worldwide invasion process of Halyomorpha halys through ABC

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The brown marmorated stinkbug (Halyomorpha halys) is a polyphagous insect pest native to Asia, which has rapidly spread worldwide causing extensive damage to global agriculture. Investigating the genetic diversity among H. halys populations is essential to understand the patterns of colonization and invasion history of local populations. Analyses based on mtDNA indicate multiple invasions from Asia, as well as serial introductions within invaded countries, but the colonization routes are still debated. Recently genomic data (ddRAD) from multiple worldwide populations of H. halys have been published, and preliminary analyses suggested a complex pattern of invasion. In this study we re-analyze published ddRAD raw sequences from worldwide populations of H. halys to better elucidate the colonization process. We assessed the genetic diversity in the native populations identifying a genetic differentiation within China and the Japanese population forming a separated genetic cluster. Furthermore, we observed a complex pattern of population structure in the invaded countries, highlighting the occurrence of multiple colonization waves. We explicitly tested alternative colonization hypotheses using Approximate Bayesian computation. Our results support a first dispersal from East China to North America, followed by a second diffusion to Europe. These results underline the importance of demographic inference through genome-wide data to investigate biological invasions.

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Section n. 7. General genetics and genomics

The evolutionary conserved chromatin organization in the HERC2/OCA2 locus, involved in the eye color

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Currently, the strongest genetic influence on eye color variation is exerted by HERC2/OCA2 locus, where the intronic SNP rs12913832, in HERC2 gene, interact with the OCA2 promoter contiguous gene via chromatin-loop organization, modulating the transcriptional activity of OCA2, directly responsible for the color eye pigmentation. Since the influence of the rs12913832 SNPs in pigmentation variation is well established in different human populations, here we analyzed the eye color variation in a sample of Sicilian population. Moreover, we highlighted the organization of the chromatin loops, by Hi-C analysis, of the HERC2/OCA2 locus which forms two contiguous chromatin loops. This region is also highly evolutionary conserved, as obtained by analysis of the syntenic regions in other vertebrate species. The relevance of a single nucleotide variation, determining the activation/inactivation of the OCA2 gene, could indicate that the organization of chromatin plays a decisive role in the correct activation of this specific gene. This finding must also be considered in its general relevance, namely if this type of polymorphism (located on an intron) is relevant in the regulation of other genes, the extensive use of NGS analyzes of exonic sequences in panels of tens or hundreds of genes, used for diagnostic purpose in complex diseases, may not be sufficient, lacking the data of intronic sequences.

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Section n. 7. General genetics and genomics

Analysis of the role of the nuclear lamina in genomic stability

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The importance of genomic stability in preventing the development of human diseases, such as cancer and neurodegeneration, has received increasing attention in recent years. Lamin is one of the major nuclear scaffolding proteins critical for maintaining the shape and architecture of the nucleus. Moreover, lamina can also act as a chromatin binding site. In addition, lamina is involved in essential nuclear functions. It plays a significant role in influencing the DNA repair pathway, as demonstrated by the accumulation of DNA damage in laminopathies caused by mutations in LMN genes. This role could, therefore, influence therapy-induced responses and acquired resistance in cancer. To study how lamina modulates the response to DSB damage, we have generated an MCF10A cell line expressing a fusion protein consisting of the restriction enzyme AsiSI and a modified estrogen receptor hormone domain. Exposure of the cells to 4-hydroxytamoxifen results in nuclear accumulation of the AsiSI-ER protein and rapid induction of approximately 150 sequence-specific DSBs throughout the genome. In this cell line, we have generated a knock-out of LMNA gene and a control cell line. Thanks to the possibility of generating damage in a site-specific way, this cellular system allows us to study and compare the recruitment of DNA repair factors at specific DSBs using ChIP-based approaches on two lines, with and without lamina.

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Section n. 7. General genetics and genomics

Satellite-free centromere formation by centromere repositioning in Malayan tapir

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Centromeres are epigenetically specified by the histone H3 variant CENP-A and typically associated with highly repetitive DNA (satellite DNA).
The order Perissodactyla includes three extant families: Tapiridae, Rhinocerotidae and Equidae. Equid karyotypes underwent rapid evolution. We demonstrated that, in this family, several centromeres are completely devoid of satellite DNA and emerged following centromere repositioning (shifting of centromere function without chromosome rearrangements) or centric fusion. The karyotypes of Tapiridae and Rhinocerotidae remained quite stable and similar to the hypothetical ancestral karyotype. The only exception is the Malayan tapir (Tapirus indicus) whose karyotype was restructured through a series of fusions.
We identified by ChIP-seq with an anti-CENP-A antibody two satellite-free centromeres in the Malayan tapir. These centromeres are located on chromosomes TIN4 and TIN15 and likely emerged from centromere repositioning. TIN4 and horse chromosome 11 (ECA11) are colinear and carry a satellite-free centromere in orthologous positions. Given the evolutionary distance between the Tapiridae and Equidae families, these results suggest the presence of a hotspot for neocentromere formation on TIN4/ECA11 chromosomes. In conclusion, the exceptional plasticity of centromeres is present in other Perissodactyla besides equids.

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Section n. 7. General genetics and genomics

The precious contribution of genetics to increase knowledge on marine species: the case of Chamelea gallina

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In the Adriatic Sea, the bivalve mollusc Chamelea gallina represents one of the most important fishing resources and it experienced a sharp decline in the last two decades, due to sudden changes in the coastal environment. Here I present results obtained on the biological status of C. gallina and the genetic diversity of its wild populations using a multidisciplinary approach. On one side, RNA-Seq allowed us to highlight an extraordinary aptitude of C. gallina to cope different environmental conditions in which it can reprogram gamete emission as function of the nutrient availability, a behavior that we defined as “opportunistic”. On the other side, employing ddRAD-Seq, we pointed out that stock reduction exhibited by this species seems not to have negatively affected its genetic diversity. Indeed, a compensatory role on local fluctuation played by the high larval dispersal rate emerged from our analyses. Moreover, sequencing the mantle transcriptome of clams collected from sites characterized by different salinity and food availability, we evidenced a different shell mineralization behaviour. Therefore, this clam can modulate the expression of genes encoding biomineralization-related proteins as function of abiotic factors. In line whit these premises, it is clear the necessity to couple genetics with classical methodologies employed to monitor this species to fully understand its biological status and thus to adopt science-based management plans to preserve this precious fishery resource.

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Section n. 7. General genetics and genomics

Differential expression of genes involved in biotic and abiotic stress in Raphanus plants collected in urban and extra-urban areas

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Intensive anthropic activities affect natural ecosystems and cause biotic and abiotic stress in the current global urban expansion. A research program on urban biodiversity is essential to improve our understanding of the ecology and evolution of species that live in urban environments, as well as their capabilities to adapt and establish in urban settings. We selected the Raphanus species as a biological target to evaluate whether urban and environmental stress caused changes in plants' natural conditions and gene expression. Raphanus has crucial agronomical value and can grow in many areas under multiple conditions. In the province of Naples, we collected Raphanus raphanistrum samples from eight locations with different levels of urbanization and distinctive soil types (tuff, limestone, lava). In order to investigate how urban environments affect plant growth and development, we examined the expression profiles of some genes from the CCT family involved in abiotic stress responses in leaf tissue. Furthermore, the analysis of the leaf surfaces of collected plants revealed a different herbivory index. For this reason, we tested the expression levels of the IQD1 gene implicated in glucosinolate metabolism, activated as a defence against attacks by pathogens and herbivores.

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Section n. 7. General genetics and genomics

New insights on the balance between transposable elements and their silencing mechanisms in ray-finned fish environmental adaptation

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Transposable elements (TEs) are characterized by the ability to insert themselves in novel genome locations. However, their transposition activity can have negative effects as altering or disrupting genes and creating genome instability. The host genome evolved various mechanisms of control and silencing of TEs using small RNAs and Kruppel-associated zinc finger box proteins that recruit proteins of the Nucleosome Remodelling and Deacetylase (NuRD) complex, increasing the heterochromatin level. Recently, a huge number of works has been published reporting that the transposition activity seems to be influenced by abiotic factors suggesting an important role in regulating mechanisms for adaptation to specific environmental conditions. It is interesting to study teleosts for their adaptation to different ecological niches and responsiveness to environmental changes. The TE genome content showed a link with environmental conditions in migratory species. Moreover, TE transcriptional activity and systems involved in their silencing, in relation to abiotic changes, as salinity and temperature, revealed for the first time a TE tissue-specific activation that might favour species adaptation to environmental changing conditions. Finally, the interaction of proteins that might be involved in the recruitment of NuRD system in fish were tested by docking simulations and in vitro by co-immunoprecipitation assay supporting the functioning of this system also in actinopterygians.

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Cytological defects in brain cells of a Drosophila model of taupathy

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In the Drosophila model, taupathic phenotypes can be reproduced by the tissue specific overexpression of the human Tau protein under the control of the UAS/GAL4 binary system. In recent years, several papers focused on many aspects of neurodegenerative taupathies, such as oxidative stress, mitochondrial damage and behavior alterations. We recently turned our attention on possible pathological phenotypes expressed at cytological level in taupathic larvae. Remarkably, we found that hTau overexpression in Drosophila melanogaster nervous system resulted in altered chromosome segregation, diploid chromosome number alterations, telomeric fusions and, most frequently, in chromatin morphological defects. These latter prompted us to deeply investigate chromatin structure and organization. We showed that two heterochromatin components, protein HP1 and the histone modification H3K9me(3), appeared ectopically distributed in both neuroblast nuclei and salivary polytene chromosomes of taupathic larvae. To investigate if the altered localization of these heterochromatin markers has also a functional outcome, we focused on position effect variegation (PEV), which depends on the spreading of heterochromatin and is influenced by the availability of heterochromatic proteins. In animals combining taupathic and variegating genotypes, PEV resulted strongly suppressed, demonstrating that tauopathy is also able to alter both structural and functional heterochromatin organization.

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Section n. 7. General genetics and genomics

Effects of UV radiation on the model organism Drosophila melanogaster

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UV radiation represents a danger for human health as it induces deleterious effects on cell homeostasis. UV rays damages biological macromolecules, especially DNA. DNA photo-products cause alterations in transcription and replication and can indirectly generate DNA breaks. In addition, UV radiation causes oxidative stress further undermining the maintenance of genomic stability. We present preliminary results on the effects of UV radiation on the model organism Drosophila melanogaster. Changes in fly physiological parameters were analysed after treatment with UV and compared with data from untreated flies. We found that white[1118] mutant (w[1118]) exhibits increased sensitivity to UV radiation especially at high doses of UVC rays respect to control Or-R. Flies bearing w[1118] mutation were isolated by Thomas Morgan in the 1918 and were fundamental to postulate chromosomal inheritance theory and to study position effect variegation. w gene is the fly counterpart of human ATP-binding cassette ABCG2 transporter, a protein with a role in Multi Drug Resistance. The peculiar behavior of w mutants in response to UV radiation will be discussed. Moreover, worldwide laboratories used the w strain as a genetic background to create transgenic flies and as a control strain. Our preliminary results indicate that loss of w reduces fly capacity to cope with UV induced stress and raise the question whether w mutant represents a reliable control for researches using Drosophila melanogaster

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miR-210 is essential to retinal homeostasis in fruit flies and mice

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miR-210 is one of the most evolutionarily conserved microRNAs. It is known to be involved in several physiological and pathological processes, including response to hypoxia, angiogenesis, cardiovascular diseases and cancer. Recently, new roles of this microRNA are emerging in the context of eye and visual system homeostasis, as well as in the regulation of circadian rhythms. In particular, miR-210 loss has been shown to lead to a progressive retinal degeneration in Drosophila melanogaster, which seems to be related with lipid droplets accumulation and alterations in lipid metabolism. However, the possible conservation of miR-210 knock-out (KO) effect in the mammalian retina remained to be investigated. Here we provide the first morphological characterization by confocal immunofluorescence and transmission electron microscopy and gene expression in the retina of miR-210 KO mice. We demonstrated for the first time the photoreceptors degeneration in miR-210 KO mice, even if there is no evidence of an involvement of lipid metabolism, as already demonstrated in miR-210 KO flies, suggesting a different mechanism of degeneration in mice than in Drosophila. Further studies will pave the way for a complete understanding of the functional role of miR-210 in the maintenance of the proper homeostasis of the mammalian visual system.

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The genetic landscape of North Africa as depicted by a massive survey of mitogenomes

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The genetic landscape of North African (NA) populations is the result of the introgression, ancient and recent movements of people as well as periods of isolation. However, source populations of these genetic contributions and the admixture events that made up the NA's genetic background at different times are still unclear. With the aim of increasing the knowledge of the genetic ancestry and history of NA populations, a dataset of 551 North African complete mitochondrial genomes (467 modern – 238 obtained during this research – and 84 ancient) was built and compared to both sub-Saharan (135 new modern samples) and Eurasian mitogenomes.
Three genetic main contributions were identified: a sub-Saharan component ascribable to the initial peopling of NA and/or to the expansion of Palaeolithic African populations that moved northward; an Eurasian component, possibly linked to late-Glacial expansions from refuge areas and/or more recent historical movements; and an ancient NA component related to a pre-Neolithic back migration from western Eurasia towards Africa. For these three major contributions, Bayesian skyline analyses revealed episodes of population growth, one of which occurred during the Holocene suggesting that the last Green Sahara could have played an important role in shaping the female-inherited NA mtDNA variability. This is the first evidence that human dispersals associated with the last Green Sahara also involved females.

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Translational Readthrough-Inducing Drugs (TRIDs) as a possible strategy to rescue LRBA protein functionality in the premature termination codon (PTC) UGA in human fibroblasts LRBA R1683X/R1683X.

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11% of the inherited genetic disorders are caused by nonsense mutations, which are due to a single-base substitution in the mRNA bringing to the onset of a PTC in codifying sequence. This leads to the premature termination of the protein translation and the production of a truncated and non-functional protein, with severe physiological consequences.
One of the most promising strategies to overcome this defect relies on the translational readthrough (TR) of PTCs.
Herein, the present study is focused on the use of three new optimized lead drugs classified as TR inducing drugs (TRIDs), namely NV848, NV914 and NV930.
Particularly, this TRIDs have been tested on human primary fibroblasts harbouring the nonsense mutation c. 5047 C>T (R1683*) in the LPS Responsive Beige-Like Anchor (LRBA) gene, causing a rare Primary Immunodeficiency disease (PID). The activity of the three compounds and their efficiency have been evaluated in this UGA-stop codon genetic context, by classical molecular approaches.
Besides, knowing that the readthrough of UGA PTC often induce Trp, Arg or Cys insertion, in-silico structure assembly simulations have been performed to predict protein structure of the corrected proteins.
Finally, mRNA next-generation sequencing (NGS) has been carried on, verifying the fidelity in the transcription under the treatment, to exclude any eventual interference by molecules, showing the correct production of the transcript.

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A comprehensive picture of the genetic history of Iran

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Ancient genome studies revealed that Iran has played a central role in the history of Eurasia being one of the major centers of agriculture invention and cattle domestication. While an "Iranian Neolithic component" is currently recognized in most of Eurasia, the genetic diversity of contemporary Iranians, especially at the Whole Genome Sequencing (WGS) level, has been poorly studied. To paint a more comprehensive picture of the genetic diversity and history of Iranian populations, we conducted novel whole genome sequencing of 87 males spanning 8 different ethnic groups and 3 language families (Indo-European, Afro-Asiatic, Turkic Altaic). After merging our WGS data with relevant available modern and ancient genomes, we found that all the Iranians, regardless of ethnicity, have a predominant pre-Neolithic autochthonous ancestry and significant ancestries from the Neolithic Anatolian farmers and Bronze Age Steppe pastoralists. Nonetheless, striking differences between ethnic groups were found due to varying gene flow from Africa (mainly in Arabs and Southern Iranians), Central Asia (Iranian Turkic-speakers and North-Eastern Persian), and South Asia (Indo-European speakers of Southern Iran). Overall, the genomic history of Iran is a multifaceted story that reflects region's position as a melting pot of diverse cultures, languages, and populations.

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Section n. 7. General genetics and genomics

Echoes from the last Green Sahara: a ghost population of cattle herders unveiled from joint whole modern genome analysis of Sahelian Fulani and ancient African individuals.

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The Sahelian Fulani are the largest nomadic pastoral ethnic group. Their origins are still largely unknown and their Eurasian genetic component is usually explained by recent admixture events with northern African groups. However, it has also been proposed that Fulani may be the descendants of ancient groups settled in the Sahara during its last Green phase (12000-5000 BP), as also suggested by Y chromosome results.
We produced 23 high-coverage (30x) whole genome sequences from Fulani individuals from 8 Sahelian countries, plus 17 samples from other African groups and 3 Europeans as controls, for a total of 44 new whole genome sequences. These data have been compared with published whole genomes from relevant populations, for a total of 814 samples. This modern dataset has been then analyzed together with relevant published ancient individuals (for a total of > 1800 ancient and modern samples). These analyses showed that the non-sub-Saharan genetic ancestry component of Fulani cannot be only explained by recent admixture events, but it is more ancient than previously reported and probably traces its origin to the last Green Sahara.
According to our results, Fulani may be the descendants of Saharan cattle herders settled in that area during the last Green Sahara. The exact ancestry composition of such ghost Saharan population(s) cannot be completely unveiled from modern genomes only, but the joint analysis with the available African ancient samples suggested a similarity between ancient Saharans and Late Neolithic Moroccans.

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Section n. 7. General genetics and genomics

Characterization of structure and evolution of the 17p11.2 region

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Genomic Structural Variants (SVs) have a central role in both evolution and pathological phenotypes. Human 17p11.2 region harbors few polymorphic inversions and five segmental duplication (SD) blocks. SDs can mediate events of Non-Allelic Homologous Recombination (NAHR), leading to duplications and deletions that can cause Potocki-Lupski Syndrome or Smith-Magenis Syndrome, respectively, and other developmental delays. With our work we investigate the frequency of these polymorphic inversions in humans and trace their evolutionary history back to the divergence time between macaque (as representative of the old world monkeys) and the great apes.
Single-cell template strand sequencing (Strand-seq) on a single individual for each of the great apes plus the macaque has been used to detect species-specific rearrangements. To validate the inversions detected with the change of directionality of the sequenced reads in Strand-seq data, we performed interphase Fluorescence In-Situ Hybridization (FISH) on nuclei preparations from the same individuals.
We were able to unravel the genetic structure of the 17p11.2 region in the analyzed species, revealing the presence of several inversions that occurred during our closest primates' evolutive history. Moreover, FISH experiments allowed us to detect nested-inversion that are not noticeable when considering only Strand-seq data, but for which the integration of data from different analyses and previous literature has been essential, such as in the case of Pan genomes.
In conclusion, our study highlights the complexity of the 17p11.2 region and clarifies its evolutive history, helping to trace the origins of SVs that are causative of human pathological phenotypes.

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ASAP - ASsessing Ancestry through Principal component analysis

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The history of human populations has been characterized by admixture events that are reflected in the genome of each population. Therefore, the evaluation of the ancestral components of human populations could be crucial to fully characterize the history of our species, which could also embed relevant information, useful to develop and design efficient medical studies and treatments.
Although many algorithms aiming to infer the population's genetic composition have been developed, most of them are characterized by poor reliability when samples with high missingness rate are analyzed, as is often the case for ancient DNA data.

It has been recently shown that F-statistics, harnessed by qpAdm to assess ancestry, are strongly correlated to PCA (Principal Component Analysis), a widely used method in population genetics to infer the genetic variation among populations. Based on this, we propose to leverage on PCA and NNLS (Non-Negative Least Squares) to assess the ancestral composition of admixed individuals. We assessed and tuned our approach on simulated data, including variable missing rate, pseudo-haploid samples and different projection strategies to mirror the quality of ancient DNA.

Our results show that the method we propose, ASAP (ASsessing Ancestry through Principal component analysis), has high accuracy and reliability, similar (and in some cases even better) to that obtained with other already available methods. Thus, we present a useful tool to assess ancestral compositions of admixed individuals/populations with good accuracy also for ancient samples and without the need to predetermine the proxy sources.

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Section n. 7. General genetics and genomics

An emergent role of p14/19Arf in pancreatic differentiation

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Although p14/p19Arf is best characterized as one of the most relevant oncosuppressor, recent evidences underline an emergent role in the context of differentiation and control of stem cells self-renewal. In fact, p19Arf has been shown to be transiently expressed in a precise time window during mouse male germ cell and eye development as well as during in vitro keratinocytes differentiation. Furthermore, p14/p19Arf must be silenced to generate iPSC and to maintain the self-renewal capacity of stem cell in physiological conditions. Altogether, these evidences places p19Arf at the crossroad between cancer and differentiation. In line with this, we recently demonstrated that Arf intracellular levels increase during in vitro differentiation of mouse ESC toward Pancreatic Progenitor Cells (PPCs). To investigate Arf's role in this process we generated genetically engineered mESCs knock-out for p19Arf by using the CRISPR-Cas9 technology. Intriguingly, the preliminary characterization of ARF null clones underlines a partial impairment in the differentiative process toward PPCs. Molecular and cytological characterization of clones' phenotypes will allow us to define Arf's role in pancreatic progenitor cells homeostasis.

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Section n. 7. General genetics and genomics

Dynamics of microbiome composition during anaerobic digestion of different renewable resources.

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Energy production from renewable resources is one of the biggest challenges of the last decade and is one of the strategic goal of sustainable development within the Agenda 2030. Anaerobic digestion of biomasses is a sustainable waste management strategy for the synthesis of biogas, a biofuel that reduces carbon dioxide emissions.
Fermentation of grape pomace, one of the most abundant waste products from agro-food industry, has a low yield in biogas production because of the high content of lignocellulose. In contrast, biomass from cereal grains is an excellent substrate but its use enters in the food-energy dilemma. Aim of the research was to investigate the fermentation dynamics of these two resources by analysing the whole genome of the microbiota (WMS) sampled at different stages of fermentation. Taxonomical classification was performed by BLAST combined with MEGAN6. For statistical analyses STAMP and LEfSe were applied.
This initial analysis highlights how the two substrates significantly differ for Peptococcaceae bacterium and Defluviitoga tunesiensis, distinctive of the cereal community, and for Hungateiclostridium saccincola and Bacteroidetes bacterium, characterizing grape pomace. Also, two clades of archaea, Methanoculleus bourgensis and Methanobacterium spp., typified respectively cereal grain and grape pomace samples.
To better define the differences in the two microbiotas, WMS data will be further analysed with the recently upgraded version of MetaPlhAn (v4.0).

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Section n. 7. General genetics and genomics

Functional genomic studies to rescue the clinical defects of PRUNE-1 syndrome.

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Neurodevelopmental disorder with variable brain anomalies (NMIHBA, #617481) is a complex incurable pediatric disease, caused by homozygous/composite heterozygous mutations in PRUNE1 locus (1q21.3).
PRUNE1 encodes for an exopolyphosphatase whose main role consists in shortening inorganic polyphosphates with emerging roles in neuronal plasticity.
To date, n.64 patients have been diagnosed with NMIHBA, with c.G316A (p.D106N) being the most occurring genetic variant (23.44%).
Omics and haplotype analyses suggested a founder effect for p.D106N variant, established in India and then moved to Mediterranean basin, including Italy.
We have generated a biobank of primary fibroblasts from NMIHBA-affected patients and healthy-carriers. Genotype/ phenotype correlation studies performed on these cells and CRISPR/Cas9-engineered retinal pigment epithelium cells, showed proliferation defects with alterations during cytokinesis phase. Genetically engineered yeast cells expressing p.D106N mutation in the homolog PPX show similar cytokinesis defects.
Further, the phenotype showed by genetically engineered mice with p.D106N mutation in murine mPRUNE1 locus is embryonic lethal (E10.5), with proliferation defects in neural precursor cells.
Ongoing experiments are based on administration of nanoliposomes-containing wild-type PRUNE1 RNA in heterozygous pregnant mice aimed to “rescue” the proliferation defects in vivo. These results will have the final goal to bring this therapeutic approach in human.

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Section n. 7. General genetics and genomics

A selective force driving metabolic genes clustering

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The evolution of operons has puzzled evolutionary biologists since their discovery and, to date, many theories have been proposed to account for their emergence and spreading. The presence of several plausible hypotheses dealing with operon emergence/evolution/spreading in extant genomes are indicative of the absence of a universal causal factor for this evolutionary process. Here, we argue that the way in which DNA replication and cell division are coupled in microbial species introduces an additional feature in determining the clustering of functionally related genes on chromosomes. We interpret this as a preliminary and necessary step in operon formation. Specifically, we start from the observation that bacterial species can accumulate several active replication forks by a partial decoupling of DNA replication and cytokinesis, which can lead to differences in copy numbers of genes that are found at distant loci on the same chromosome arm. We provide theoretical considerations suggesting that, when genes of the same metabolic process are far away on the chromosome, changes in the number of active replication forks result in perturbations to metabolic homeostasis. By formalizing the effect of DNA replication on metabolic homeostasis based on Metabolic Control Analysis, we show that the above situation provides a selective force that can drive functionally related genes at nearby loci and that therefore could explain gene clustering and operon formation. Finally, we confirmed that, in present-day genomes, this force is significantly stronger in those species where the average number of active replication forks is larger and quantify the theoretical contribution of this feature on the distribution of extant gene clusters and operons.

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Section n. 7. General genetics and genomics

Exploring the interconnections between DNA-RNA hybrids and the DNA damage response

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DNA-RNA hybrids are normally generated at highly transcribed regions, centromeres and telomeres and participate in a number of physiological processes such as transcription, immunoglobulin class switching and epigenetic modifications. When their homeostasis is perturbed, they can become source of DNA damage and genomic instability, a hallmark of cancer and other genetic diseases. How these structures affect genome stability is not completely understood.
The aim of this project is to investigate how DNA-RNA hybrids can activate the cellular response to DNA damage and to study the interplays between DNA-RNA hybrids metabolism and the DNA damage response. We combine mutations in genes involved in DNA-RNA hybrids processing, as those encoding the Sen1/Senataxin helicase or the RNase H ribonucleases, with mutations in genes involved in the DNA damage response in Saccharomyces cerevisiae. This approach allowed us to identify both negative and positive genetic interactions between Sen1, RNase H and nucleases that operate in the DNA damage response.
Since Senataxin and the DNA damage response genes are mutated in tumours and genetic diseases, understanding the molecular mechanisms at the basis of these genetic interactions could be useful for the identification of pathogenesis mechanisms and of new targets and strategies for therapies.

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Section n. 7. General genetics and genomics

Interplays between checkpoint kinases, nucleases and helicases in the DNA damage response

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DNA double strand breaks are the most cytotoxic lesions that threat our genome and could lead to genome instability if they are not properly repaired. They are sensed by the protein kinase ATM/Tel1 which orchestrates a complex genetic network that arrests the cell cycle through the activation of a checkpoint pathway and repairs the damage. Tel1/ATM represents a relevant target for cancer therapy because either germline or sporadic ATM mutations were identified in different tumors. To provide novel targetable proteins in anticancer therapy in combination with ATM mutations/inhibition, we searched for novel synthetic cytotoxic interactions with Tel1/ATM mutations in the budding yeast Saccharomyces cerevisiae. We performed a genomic screening searching on one hand for mutants that exacerbate the sensitivity to DNA damaging agents of cells lacking Tel1, and on the other hand for mutants that can suppress this sensitivity. Among the identified genes, we found nucleases and helicases involved in different DNA repair pathways, thus suggesting that the activity of these enzymes becomes crucial for the cells to survive to DNA damage in the absence of Tel1. Understanding the molecular mechanism underlying these genetic interactions could contribute to unravel novel Tel1/ATM interplays in the DNA damage response and to discover new therapeutic targets for anticancer treatments.

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Section n. 7. General genetics and genomics

A chromosome-level reference genome and pangenome for barn swallow population genomics

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The lack of reference genomes of high accuracy, completeness, and contiguity often limits insights into the genomic evolution of non-model organisms. We present a new chromosome-level, karyotype-validated reference genome for the barn swallow (Hirundo rustica), a charismatic migratory bird with six recognized subspecies. First, we used this reference to generate a comprehensive catalog of genetic variants using all available data for the species. Next, we conducted a resequencing project involving several individuals from three Eurasian barn swallow populations to conduct association studies with behavioral traits. Thanks to High-Fidelity long reads, we identified SNPs and structural variants and analyzed genetic variability within and among populations. We also used our reference to align 10x Genomics data of barn swallows from three sites in Ukraine with different levels of contamination due to the Chernobyl accident, aiming at identifying a putative set of mutations potentially induced by environmental radiation. Finally, we generated the first pangenome for the species comprising the reference genome and 5 H. rustica individuals sequenced with HiFi long reads. We expect the pangenome to reduce bias towards a single reference genome, and we plan to test its use for read mapping and variant calling, exploiting the potential of pangenome graphs for population genomics.

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Section n. 7. General genetics and genomics

Physical activity, sedentary behavior and pancreatic cancer risk: a Mendelian randomization study.

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Pancreatic cancer is currently the seventh leading cause of cancer death worldwide. Understanding whether modifiable factors increase or decrease the risk of this disease is central to facilitating primary prevention. Several epidemiological studies have described the benefits of physical activity, and the risks associated with sedentary behavior, in relation to cancer.
This study aimed to assess evidence of causal effects of physical activity and sedentary behavior on pancreatic cancer risk. We conducted a two-sample Mendelian randomization study using publicly available data for genetic variants associated with physical activity and sedentary behavior traits, and genetic data from the Pancreatic Cancer Cohort Consortium (PanScan), the Pancreatic Cancer Case-Control Consortium (PanC4) and the Finngen study for a total of 10,018 pancreatic cancer cases and 266,638 controls. We also investigated the role of body mass index as a possible mediator between physical activity and sedentary traits and risk of developing pancreatic cancer.
We found evidence of a causal association between genetically determined hours spent watching television and increased risk of pancreatic cancer (PanScan-PanC4 odds ratio [OR] = 1.52, 95% CI 1.17-1.98, P = 0.002). Additionally, mediation analysis showed that genetically determined television-watching time (hours per day) was strongly associated with BMI, and the estimated proportion of the effect of television-watching time on pancreatic cancer risk mediated by BMI was 54%.
This study reports the first Mendelian randomization-based evidence of a causal association between a measure of sedentary behavior (television watching time) and risk of pancreatic cancer, and that this is strongly mediated by BMI.

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Section n. 7. General genetics and genomics

8-oxodG: a driver or passenger of the transcription machinery?

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8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a primary outcome of DNA oxidation, has recently been redefined as an epigenetic factor, which influences the transcription process. However, the precise role of 8-oxodG in global transcription regulation remains to be fully elucidated.
To explore the involvement of 8-oxodG in global transcription regulation, we adopted two complementary approaches. Firstly, we inhibited RNAPII activity to investigate whether the accumulation of 8-oxodG is dependent on RNAPII movement. Secondly, we reduced the genomic levels of 8-oxodG to assess the impact on RNAPII traveling.
The results of our investigation propose that 8-oxodG actively drives the transcription machinery rather than merely being a passive bystander.

Group **A** Poster n. **53**

Section n. 7. General genetics and genomics

A REM-ARF complex controls inflorescence architecture determination in Arabidopsis thaliana

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The organization of floral primordia around the main stem of the inflorescence, called phyllotaxis, follows strict geometrical constraints. The establishment of the phyllotactic pattern is a self-organizing process resulting from the combinatory effects of inhibitory fields, generated by the emerging primordia. These inhibitory fields are auxin-depleted areas, responsible for setting the spacing between successive organs initiation.
The genetic network that controls inflorescence architecture has been partially unravelled, however, the precise mechanism that controls the timing and spacing between new flower initiation at the meristem level is still unknown.
The transcription factors REM34 and REM35 are expressed from the earliest stages of the reproductive development of Arabidopsis, and we found that single and double rem34 rem35 mutants displayed an altered phyllotactic pattern, with divergence angles distributed around three main values: 90°, 140° and 180°.
REM35 interacts with REM34 and the Auxin Responsive Factor 7 (ARF7) and ARF19, already characterized in the lateral root formation. We showed that arf7 arf19 loss-of-function mutants display a mutated phyllotactic pattern, phenocopying rem34 and rem35. REM35/35 partially share direct targets with ARF7/9 as PUCHI and LBD18, two boundaries-expressed genes. Interestingly, both rem34 rem35 and arf7 arf19 mutants show enlarged inflorescence meristems, in which the expression pattern of PUCHI is reduced.
These findings suggest that REM34 and REM35 collaborate with ARF7 and ARF19 in the control of inflorescence architecture establishment and boundaries specification in the IM/FMs.

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Section n. 7. General genetics and genomics

IN VIVO EXPOSURE TO MICROPLASTICS AND CAR TIRE. CHROMOSOMAL ALTERATIONS IN FRESHWATER FISHES

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Research on microplastics (MPs) is one of the most trending topics in science because of the ubiquitous nature of these small (<5 mm) particles, found in every environmental matrix with different concentrations.
The aim of the study was to investigate the possible chronical effect of MPs of different plastic polymers and car tires, mixed with a coarse sediment on the freshwater fish European perch (Perca fluviatilis).
The exposure times were 4 and 7 months. Chromosomal damage was assessed on peripheral blood erythrocytes by Cytome assay. Ethoxyresorufin-O—deethylase (EROD), Glutathione reductase (GR) and Acetylcholinesterase activities were also investigated.
A statistically significant induction ($p<0.05$) of chromosomal aberrations (dicentric chromosomes) after 7 months in co-exposed specimens was found. Higher ($p<0.05$) frequencies of 8-shaped nuclei, a decrease in cell proliferation and an increase of apoptotic cells were also assessed in the same group after 7 months. No statistically significant increases of micronuclei were detected. Preliminary results showed enhanced levels of EROD activity after chronical exposure.
In conclusion, low levels of chemical exposure, prolonged in time, induce visible effects on aquatic organisms, both in terms of chromosomal stability loss and antioxidant capacity deficiency, suggesting that more studies are needed to investigate effects of MPs and car tire in edible organisms and possible consequences for their predators, including humans.

Group **A** Poster n. **55**

Section n. 7. General genetics and genomics

Dysregulation of NF–Y splicing drives metabolic rewiring and aggressiveness in colon cancer

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NF-Y is an evolutionarily conserved transcription factor that binds specifically to the CCAAT elements of eukaryotic genes, most of which frequently deregulated in cancer. NF-YA, the regulatory subunit of the NF-Y complex, has two isoforms generated by alternative splicing, NF-YAl and NF-YAs, which differ in the transactivation domain.
Transcriptomic data from The Cancer Genome Atlas (TCGA) database highlighted a significant increase in the expression of NF-YAs at the expense of NF-YAl in colorectal cancer (CRC), compared to healthy tissues. Despite this, high NF-YAl levels predict lower patients' survival and distinguish the mesenchymal molecular subtype CMS4, which is characterized by the worst prognosis.
Through the analysis of 3D cellular models, we demonstrated that altered expression of genes related to extracellular matrix and epithelial-mesenchymal transition sustains enhanced migratory and invasive behavior of NF-YAl-transduced cells. Moreover, the integration of metabolomics, bioenergetics and transcriptional analyses demonstrated a direct role for NF-YAl in metabolic flexibility of cancer cells that adjust their metabolism in response to environmental changes to potentiate migration. The zebrafish xenograft model confirmed the metastatic potential triggered by NF-YAl in CRC cells.
Altogether, our data highlight the transcriptional role of NF-YAl in CRC aggressiveness and suggest splice-switching strategies to hinder NF-YAl-induced metastatic dissemination.

Group **B** Poster n. **1**

Section n. 4. New sequence technologies and pangenome analysis

Pangenome-based inference of structurally complex haplotypes from short-read data

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Background:
Pangenome graphs model genomic variation of multiple individuals. Genotyping is inferring the alleles of a DNA sample from sequenced reads, which can be enhanced by using a pangenome graph instead of a single genome as reference.
Method:
Here we present cosigt, a novel computational workflow and software for genotyping structurally complex haplotypes from short-read sequencing data using a pangenome graph.
For a given short-read sample and region of interest, cosigt (1) aligns short reads to the nodes in the graph and (2) compare the coverage of the short reads over nodes with the coverage of each diploid combination of haplotypes from the reference graph by means of cosine similarity, picking the combination with the highest similarity score.
Results:
We benchmarked cosigt on the AMY1 locus using short-read data from 23 1000G individuals with matching haplotypes in the HPRC pangenome graph. Comparison of the expected haplotype structure with that predicted by cosigt revealed that our method could precisely genotype ~86% of the evaluated samples, achieving 96% accuracy at matching structural haplotype clusters inferred from a graph-based haplotype similarity. We also applied our workflow on a diverse dataset with > 3000 individuals from 1000G, HGDP and SGDP and we found that AMY1 gene copies in the haplotypes predicted by cosigt precisely matched the copy numbers predicted by the sequenced reads in more than the 95% of the samples analyzed.

Group **B** Poster n. **2**

Section n. 4. New sequence technologies and pangenome analysis

A new comparative analysis of DNA fragmentation by TUNEL assay: an in vitro model for the grading of genomic instability

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DNA damage is one of the most important effects induced by chemical agents. Here we report a comparative analysis of DNA fragmentation on different cell lines using the TUNEL assay, generally used to highlight DNA fragmentation that can be caused by apoptotic phenomena.
Our approach combines cytogenetic techniques and analysis of fragmented DNA from cellular structures in suspension, recovered from medium culture.
The cells were treated with resveratrol and doxorubicin, in single and combined treatments. TUNEL test was performed to detect any fragmentation point on single chromosomes, whole nuclei and other cellular structures.
Astrocytes showed DNA damage in condensed nuclei and structures in suspension. Caco-2 cells had fragmented nuclei only after doxorubicin treatment. Control samples had fragmented chromosomes, indicating DNA damage in replicating cells. MDA-MB-231 cells showed nuclear condensation and DNA fragmentation, especially when treated with resveratrol, along with fragmented DNA in structures in suspension.
This model proved to be able to perform a grading of genomic instability (GI). Astrocytes show a hybrid level of GI. Triple negative MDA- MB-231 cells attain an intermediate degree of GI, probably to ensure the metastatic invasion. We assume that these cells acquire the so-called "mutator phenotype". Caco-2 cells have a high instability level, having no DNA repair mechanisms activated, to ensure cell survival but not acquiring the metastatic characteristics.

Group **B** Poster n. **3**

Section n. 4. New sequence technologies and pangenome analysis

Host-microorganism interactions in obesity and colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer and the second cause of cancer-related mortality in the world. It is well known that the lifestyle has a crucial role in CRC development. The current evidence indicates that obesity increases the risk of carcinogenesis including CRC.
Notably, obese subjects and CRC patients share common features: oxidative stress, chronic inflammation, immunosuppression, mitochondrial (mt) dysfunction, adipose tissue deregulation as well as gut dysbiosis. A plethora of studies has characterized the bacterial component of the gut microbiome, but what is known about the viral component? Moreover, the role of the oral microbiome has been only poorly investigated.
In order to address these questions cohorts of severe obese subjects, CRC patients (at different tumour stage) and healthy individuals have been enrolled, and stool, saliva, blood and tumour tissues have been collected. Preliminary data show an increase of Human Polyoma and beta Papilloma Virus infections in both obese and CRC patients versus the control counterpart as well as an abundance of common and multiple infections. An interesting modulation of microbiome composition has emerged too. Markers of oxidative stress/inflammation and cell free circulating mtDNA have been analysed in plasma samples and correlated with clinical, anthropometric and lifestyle parameters. All together these data might lead to the identification of predictive and prognostic indicators of CRC risk.

Group **B** Poster n. **4**

Section n. 4. New sequence technologies and pangenome analysis

Comparative analysis of Presence-Absence gene Variations in five hard tick species: impact and functional considerations

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Tick species are vectors of harmful human and animal diseases and their expansion is raising concerns under the global environmental changes scenario. Ticks host and transmit bacteria and viruses making the understanding of host-pathogen molecular pathways critical to develop effective disease control strategies. Despite the considerable sizes and repeat contents of tick genomes, individual tick genomics is perhaps the most effective approach to reveal genotypic traits of interest. Presence-Absence gene Variations (PAVs) can contribute to individual differences within species, defining dispensable genes carried by subset of individuals only, possibly underpinning functional significance at individual or population-levels. We exploited 350 resequencing datasets of individual Dermacentor silvarum, Haemaphysalis longicornis, Ixodes persulcatus, Rhipicephalus microplus and Rhipicephalus sanguineus hard-tick specimens to reveal the extension of PAV and patterns of dispensable genes among individuals and, comparatively, between species. We traced 550-3,346 dispensable genes per species, mostly referring to transposable elements. Moreover, we reconstructed the pangenomes of these 5 tick species, obtaining a range of 5.3-7 Mb per species not included in the respective reference genomes. Finally, we detected relevant differences in the extension and distribution of dispensable genes among species, possibly underpinning functional significance.

Group **B** Poster n. **5**

Section n. 4. New sequence technologies and pangenome analysis

PangenomEX: A new era of isogenomic reference human genomes

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The recent human pangenome showcased near-complete Telomere to Telomere (T2T) diploid genomes from 47 genetically diverse individuals. Several genomic loci display heightened sequence polymorphism, with variation across the cohort especially relating to repetitive elements. In line with this divergence, the current use of single reference genomes may be unsuccessful to support the correct analyses of reads coming from experiments done in different cell lines or across a population. Here, we coined the term isogenomic to describe a novel approach in human genomics to improved omics analyses using cell lines' matched reference genomes. We present the first example of such isogenomic reference using T21T de novo diploid assembly of one of the most widely used primary laboratory cell line, RPE1, derived from the human retinal epithelium. We show how the use of this isogenomic reference genome to analyze omics data including CUT&RUN, ChIP-seq, and HiC and demonstrate drastic improvements in alignments and reduced biases for peaks calling at polymorphic genomic loci. Finally, we are excited to introduce to the AGI the PangenomEX, launched in May 2023 – an open collaborative sister initiative to the HPRC toward a Pangenome of EXperimentally relevant human cell lines.

Website: https://sites.google.com/uniroma1.it/giuntalab

Group **B** Poster n. **6**

Section n. 4. New sequence technologies and pangenome analysis

Several functionally distinct Paenibacillus species exist based upon pan-genome analyses of Paenibacillus polymyxa strains.

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The rapid development of sequencing technologies has led to an exponential increase in microbial sequencing data. Accurately identifying bacterial species remains a major challenge, because of extensive intra-taxon variability and the need to combine the analysis of both phenotypic and genotypic traits. In this context, pan-genome analysis offers a broad perspective to study the complete genetic information of a species. Paenibacillus polymyxa, a Gram-positive bacterium commonly found in soil and plant roots, is a well-known producer of antibiotics, such as polymyxin, and plays a relevant role in agriculture, due to its nitrogen-fixing capacity. Here, we present a robust framework for examining evolutionary and taxonomical relationships comparing genomes of strains belonging to this species available at NCBI, as well as new strains isolated and sequenced at the University of Camerino. Based on digital DNA-DNA hybridization, average nucleotide identity (ANI) estimation, and OrthoFinder, we found P. polymyxa strains to be consistently divided into four clusters with an open pan-genome. Given the high dynamism of the genomes belonging to this species, this comparative analysis provides new insight into the genomic content and variability of P. polymyxa. The analysis of genes belonging to the core genome can help in distinguishing strains with different properties, opening the possibility for a more robust and accurate classification of this biotechnology relevant bacterium.

Group **B** Poster n. **7**

Section n. 4. New sequence technologies and pangenome analysis

Analysis of genetic and epigenetic structure and variability of grapevine centromeres through the use of long read sequencing and T2T assemblies

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We produced assemblies of 3 European and Caucasian cultivated grapevine accessions, one European wild accession of the same species and one interspecific hybrid between North American wild species using PacBio HiFi and in some cases ONT long-reads and the Hifiasm software. A large fraction of the chromosomes in each accession were T2T resolved and allowed us to perform a detailed analysis of the elusive structure of the centromeres. The comparison of different centromeric haplotypes for the same chromosome has also allowed us to study the variability and the evolutionary history of these regions. The methylation analysis using ONT reads allowed us to study the epigenetic status of these regions despite their structural complexity. The structure of the centromeres was reconstructed at fine resolution and revealed the presence of highly ordered and symmetrical structures, with several families of tandem repeats, forming in some cases Megabase-sized arrays, intermixed with retrotransposons mainly belonging to the Athila and the chromovirus families of Gypsy elements. The grapevine centromeres appear to be densely DNA methylated, especially in the arrays of the most abundant tandem repeat. Extreme variability was observed in terms of structure and repeat composition both among different chromosomes as well as among haplotypes of the same chromosome and haplotypes of different accessions, attesting a very high rate of evolution of these regions.

Group **B** Poster n. **8**

Section n. 4. New sequence technologies and pangenome analysis

Generating a complete human panmitogenome

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Nicole Huber (3),
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Claudio Fiorini (5),
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Current sequencing technologies and algorithms routinely allow the generation of complete human mitogenomes. However, the use of a single individual sequence (conventionally, rCRS) as a reference could impact variant calling (especially around indels or structural variants) and might lead to underestimate the genetic variability of the population under study. Several strategies to mitigate this issue have been proposed over the years, but with limited success. Recent developments in pangenomic data structures and methods, such as those spearheaded by the Human Pangenome Project, now allow to prevent such bias by generating a variation graph from multiple sequences. This provides the opportunity to generate a single panmitogenome representative of the entire variation within a species. As part of the Human Pangenome Reference Consortium, we generated a first panmitogenome representative of the human mtDNA variability. This includes sequences selected considering available phylogenetic information as well as stringent quality criteria to represent the worldwide mitogenome variation. This should improve the variant calling and help to clarify the full extent of indels and structural variation in the human mitogenome, and will serve as a key part of a human pangenome reference system for downstream analyses.

Group **B** Poster n. **9**

Section n. 5. Single cell analysis

Understanding the origin of sex-bias in human autoimmunity by investigating X chromosome inactivation

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The major genetic difference between the human sexes is the presence of two X chromosomes in the female (XX) and one X and a Y chromosome in the male (XY). This genetic unbalance is compensated by silencing of one of the two female X chromosomes, named X chromosome inactivation (XCI). In human, a wide number of genes escape XCI and recent studies show that other genes can spontaneously reactivate. Here, we test the hypothesis that XCI escape/reactivation might underlie sex-differences in human diseases. To this, we investigate Multiple Sclerosis (MS) a neurodegenerative autoimmune disease that affects women more than men (3:1). We show that the Xi has a relaxed chromatin landscape in human T lymphocytes, as highlighted by the delocalization of XIST long non-coding RNA from the territory of the inactive X chromosome (Xi). Notably, XIST properly re-localizes onto the Xi after T cell activation but this re-localization is delayed in MS patients compared to healthy donors. High-throughput single-cell RNA sequencing reveals an expansion of specific memory T cell subsets in the females and in association with the disease. Several X-linked genes that escape XCI are among sex-differentially expressed genes, thus providing candidates for the MS sex bias. Future studies will investigate the mechanisms of XCI escape and determine sex-based functional differences in the identified T cell subsets. This will ultimately pave the way for sex-tailored therapies.

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Section n. 5. Single cell analysis

NF-YAlong isoform sustains the mesoderm specification process in mouse embryo development.

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Following the formation of a zygote by the two gametes, the transcriptional program of the new individual is progressively installed. At the heart of these processes are Transcription Factors -TFs- the driving force to activate novel waves of gene expression. Mouse Embryonic Stem Cells -mESCs- recapitulate well gene regulation features and epigenetic modifications involved in the maintenance of totipotency and differentiation to precursors of the original embryo germ layers.
NF-Y, a trimeric TF binding to the CCAAT element, is composed of three subunits, two with histone-like features, NF-YB/NF-YC, and the regulatory NF-YA, conferring sequence-specificity to the complex. NF-YA comes in two isoforms, NF-YAs and NF-YAl, produced by alternative splicing of Exon 3. The stemness of mESC has been associated to NF-YAs, while NF-YAl is expressed during differentiation process. We specifically deleted with CRISPR/Cas9 technology the 28 aminoacids of NF-YAl coded by Exon 3 in mESCs, forcing the NF-YAs expression.
Exploiting different mesoderm differentiation protocols, bulk and Single cell Rna-Seq data indicate that NF-YAl is crucial for the early differentiation process of mesoderm layer affecting the formation of mesoderm population. Through cut and run experiments, we discovered that NF-YA isoforms bind a set of isoform specific DNA binding sites and specifically interact with transcription factors to regulate the differentiation process of the three germ layers during development.

Group **B** Poster n. **11**

Section n. 5. Single cell analysis

In vitro and in vivo genotoxicity of quinoin, a ribosome inactivating protein (RIP) from quinoa seeds, in zebrafish

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In recent years, quinoa has received a great deal of attention due to its high content of essential amino acids and minerals, beneficial to human health. However, quinoin, a toxic enzyme classified as ribosome inactivating protein (RIP), recently found in quinoa seeds (Chenopodium quinoa Willd), exhibits in vitro cytotoxic action. Considering that DNA depurination and apoptosis by RIPs have already been suggested, our study aims to evaluate the in vitro and in vivo quinoin genotoxicity on zebrafish model by TUNEL technique and RAPD-PCR on both zebrafish blood cells exposed in vitro to several quinoin amounts and blood cells collected from zebrafish specimens after different days of intraperitoneal route administration of several quinoin amounts. Furthermore, since the DNA damage has been reported to be secondary to oxidative stress, we estimated the percentage of Reactive Oxygen Species (ROS) in exposed blood cells using DCF assay. Our results showed that quinoin induced ROS-mediated genotoxicity in zebrafish. The interesting data emerging from this study is a lower percentage of damage at longer quinoin treatments compared to shorter ones. This result could indicate the activation of detoxification and/or repair mechanisms, as well as the loss of protein activity caused by enzymatic digestion. Overall, quinoin can induce genotoxic damage to the zebrafish genome acting through ROS generation, suggesting that the presence of quinoin in quinoa could be harmful to the consumers.

Group **B** Poster n. **12**

Section n. 5. Single cell analysis

The influence of ancient ancestries on the immunological landscape of present-day Europeans

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Throughout human history, the immune system has undergone remarkable evolutionary changes, largely to cope with new environments and selective pressures. The genomes of modern Europeans are the result of the admixture of at least three main ancient groups. By studying how these ancestries affect the gene expression of immune cells we can learn the origins, the variation and the risks of immune diseases across groups of people.
Here, we analysed a large-scale publicly available dataset encompassing genome-wide SNP variation and PBMC single-cell RNA-seq profiles in about 1,000 donors. We combined this dataset with over 6,000 modern and ancient reference genomes to examine the genetic structure and estimate ancient contributions. Additionally, we investigated the effect of these ancestries on the immune system by testing their impact on single-cell variability.
Our findings highlight significant differences in the abundance of lymphoid cell types as the amount of ancient components from the Paleolithic, Neolithic and Bronze Age groups changes in Central European-enriched donors. Furthermore, key genes associated with MHC clusters were the most prevalent among those with an expression significantly correlated with these ancient components.
These findings pave the way to a better understanding of the role of evolution on genetic differences in the risk of disease, which informs on the molecular bases of the diseases themselves and ultimately may lead to personalised treatments.

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Section n. 5. Single cell analysis

MultiOmics Single Cell Network Embedding

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The development of single-cell multi-omics techniques provides huge new opportunities to unveil the complexity of underlying biological systems but effective tools for getting insight from data by researchers are lacking. In this context, researchers should be provided with powerful tools to master the huge amount of cancer data. Methods for the integration of multimodal single-cell data are still underdeveloped and future challenges include accounting for developing biologically interpretable integration strategies. We previously developed a tool, called MoNETA, for fast and biologically informed integration of multi-omics bulk data to be used for sample stratification.
Here we show the performances of MoNETA in the task of multi omics single cells data integration and projection. In particular, we benchmark MoNETA on three bimodal single cell assays derived respectively from bone marrow mononuclear cells, peripheral blood mononuclear cells from lung, mouse skin cells and on two trimodal single cell assays of peripheral blood monocyte cells.
Moreover, we compare MoNETA with state of the art multi-omics single cell integration methods and show how it competes with these methods in the task of cell type separation.

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Section n. 6. Genetics of aging

ADAM17 genetic variability and Alzheimer's disease risk susceptibility

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ADAM17 is a sheddase with over 80 currently described substrates that work in different physiological processes, many of which play a key role in Alzheimer's disease (AD). Only two studies analysed ADAM17 variant in AD patients with mixed results. The aim of this study was to better characterize the association between ADAM17 genetic variability and AD susceptibility.
To this purpose, a case-control association study was conducted in a Southern Italian cohort of 112 AD patients and 180 age- and sex-matched controls. Seven SNPs were selected by a tagging-approach procedure and then genotyped using RT-PCR assays. The associations between these tag-SNPs and the risk of AD were assessed by logistic regression models.
The variability of two polymorphisms (rs12692385 and rs11690078) was related to the AD onset. After adjustment for sex and APOE □4 status, carriers of the C allele of the rs12692385 had about 1.76 higher probability to develop AD than subjects who were homozygous for the T allele (p=0.029). A significant effect was also detected the rs11690078 for which subjects who were homozygous for the C allele had about 2.29 higher probability to develop AD than subjects carrying a single copy of the T allele (p=0.033)
Our results reveal a new role of the ADAM17 gene in AD from a genetic perspective and that ADAM17 gene should be considered for the genetic screening of AD.

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Section n. 6. Genetics of aging

Effects of stilbenoid compounds on the cell cycle of neuroblastoma cells

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Stilbenoids are natural phenolic compounds, present in several plants, endowed by potential therapeutic actions, and among these Resveratrol and Pterostilbene have important biological properties as antioxidant, anti-cancer, and potential anti-aging, cardioprotective and neuroprotective effects. Here, we investigated the effect of these stilbenoids on the cell cycle of the SK-N-BE cell line, before and after neuronal differentiation induced by retinoic acid, to highlight a possible deregulation of the cell cycle not only in cancer diseases, but also in neurodegeneration. The ectopic restart of a cell cycle in differentiated neurons seems related to the next cell death. The study was performed by evaluation of the expression level of cyclins, related to cell cycle progression, and by immunodetection of specific cell cycle markers. Stilbenoids showed distinct effects on cyclin expression in replicative, differentiated, and cells induced at neurodegeneration, with differences between resveratrol and pterostilbene. These two compounds at high and low concentrations in our cellular model revealed differences in expression of cyclin D1, confirming the hormetic effect of stilbenoids, that show beneficial effect at low doses but not at the higher ones. Considering the growing interest to these natural molecules, it is useful to investigate on their effects at the genomic and cellular level, for a future use as neuroprotective compounds and to a global improvement of human health.

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Section n. 6. Genetics of aging

Variability of FOXP2 and its targets CNTNAP2 and PRNP in age-related neurodegeneration: a study in Frontotemporal Dementia

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Speech and language skills have sparked the interest of scientists as one of the key traits that set humans apart from other species. The FOXP2 (Forkhead box P2) gene, firstly associated in 2001 with mendelian language disorders and thus identified as “the language gene”, codifies for a transcription factor with several targets. Expressed in different brain regions during development and adulthood, FOXP2 mutations are directly implicated in speech production and associated disorders in children, including autism. Nowadays, its role in age-related neurodegeneration drawn attention of research,. We queried about the role of FOXP2 variability in dementia by analysing a panel of 27 SNPs mapped on FOXP2 and its targets CNTNAP2 and PRNP, in a sample of 113 frontotemporal dementia (FTD) patients and 223 Italian controls. Two markers of FOXP2, rs1456029 and rs17213159, were associated with the disease, the second holding FDR correction ($p<0.05$). SNPs at FOXP2 and CNTNAP2 were associated with verbal fluency, while all three loci resulted associated with cognitive status and neurocognitive tests at a nominal p-value. Interestingly, multi-dimensional reduction analysis found a 3-order epistatic interaction among rs1456029- FOXP2, rs13045348-PRNP and rs82664-CNTNAP2 in determining the phenotype . Overall, data suggest that FOXP2 is implicated in FTD and the mutual collaboration with these two targets may represent a driving factor in the disease pathogenesis.

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Section n. 6. Genetics of aging

Association of Leucocytes Telomere Length with risk of frailty in the elderly population

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Frailty is a multidimensional syndrome that affects multiple systems and predisposes to unhealthy outcomes. It is associated with greater vulnerability to stressors and increased risk of adverse health outcomes. Hence, finding epi/genetic biomarkers with diagnostic/prognostic capacity is a major milestone to identify frailty risk. Among them, the measurement of peripheral blood telomere length is
emerging as a promising factor for frailty diagnosis.
Leucocytes Telomere Length (LTL) shows great individual variability and is associated with aging and with age-associated diseases, such as cardiovascular and Alzheimer's diseases.
The aim of this study is to evaluate the association of LTL with frailty in a cohort of older adults 250 unrelated subjects (median age 80.17±5.06) from Southern Italy. To classify the subject respect to his/her frailty level a hierarchical cluster analysis on specific geriatric parameters, including Mini Mental State Examination, Self-Reported Health Status, Activity of Daily Living
and Hand Grip strength was applied.Three clusters were considered: non frail, prefrail and frail. Data analysis, using sex and age as covariates showed that mean LTL was shorter in frail than non-frail individuals ($p=0.004$). This trend was also observed by comparing pre-frail vs non frail and pre-frail vs frail ($p=0.071$ and $p=0.056$ respectively). These findings agree with literature data suggesting telomere length as a possible biomarker for frailty.

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Section n. 6. Genetics of aging

Development and validation of a targeted and flexible epigenetic clock: from forensic purposes to clinical applications

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DNA methylation variants have been widely used as biomarkers of ageing and several mathematical models have been developed to estimate the biological age.
We developed a targeted epigenetic clock purposely optimized for the measurement of biological age. The clock includes five DNAm biomarkers strongly correlated with chronological age (ELOVL2, C1orf132, FHL2, KLF14 and TRIM59) whose DNA methylation levels were measured by using a multiplex methylation SNaPshot assay.
In healthy subjects (n=104), epigenetic age calculated using the developed clock was highly correlated with age (r=0.95). The formulated clock was validated in several phenotypes of decreased (nonagenarians and centenarians) or increased (patients with Fabry (FD) and Alzheimer disease (AD)) biological age. We found that nonagenarians and centenarians presented a younger DNAm age than their chronological age (86.0 vs 100.4). AD patients exhibit a significant accelerated ageing that was strongly correlated with disease severity. Surprisingly, while some FD patients showed a DNAm age in line with their chronological age, other FD patients showed a significant decelerated aging phenotype mainly due to an altered methylation status of FHL2 promoter.
These results show the potential of our targeted epigenetic clock as a new marker of biological age and open its evaluation in large cohorts to further promote the assessment of biological age in healthcare practice.

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Section n. 6. Genetics of aging

Premature aging in Drosophila model under cadmium-related stressful conditions

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Aging is a progressive cellular and functional decline of an organism over time. During aging, cellular damages accumulate leading to a loss of physical and mental efficiency, and an increased vulnerability to diseases as well. It is known that environmental pollutants could impact cellular signs of aging and are responsible for the high morbidity of age-related diseases. We used Drosophila melanogaster as a model to investigate the impact of the environmental contaminant cadmium, introduced with the diet, on aging-related processes. Treated flies exhibited a significant decrease of learning and memory abilities over time indicating a premature aging. In addition, specific molecular markers of aging, including transposable elements, memory and stress genes, were deregulated in the fly brain indicating a premature loss of transcriptional and/or posttranscriptional control. These data, together with the analysis of neuronal cell organization, lay the foundations to understand how environmental stress contribute to aging-related degeneration.

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Section n. 6. Genetics of aging

Genetic aspects underlying the Normocalcemic and Hypercalcemic phenotypes of Primary Hyperparathyroidism

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Hypercalcemic primary hyperparathyroidism (PHPT) is a common endocrinedisorder that has been very well characterized. In contrast, many aspectsof normocalcemic primary hyperparathyroidism (NPHPT) such as natural history, organdamage, and management are still matter of debate. In addition, both thepathophysiology and molecular basis of NPHPT are unclear. We investigated whetherPHPT and NPHPT patient cohorts share the same pattern of genetic variation in genesknown to be involved in calcium and/or bone metabolism.
Genotyping for 9 SNPs was performed by Real-Time PCR (TaqMan assays) on 27 NPHPT and 31PHPT patients. The data of both groups werecompared with 54 in house-controls and 503 subjects from the 1,000 GenomesProject. All groups were compared for allele/haplotype frequencies, on a single locus,two loci and multi-locus basis.
Preliminary results showed that the NPHPT group differed significantly at SNPs in OPG and ESR1. Also, the
NPHPT cohort was peculiar for pairwise associations of genotypes and for theoverrepresentation of unusual multilocus genotypes.
In conclusion, our NPHPT patient set harboured a definitely larger quota of geneticdiversity than the other samples. Specific genotypes may help in defining subgroups ofNPHPT patients which deserve ad hoc clinical and follow-up studies. ACKNOWLEDGMENTS: Work supported by POR FESR Lazio 2014-2020 (n. A0375-2020-36631) to P.M. and
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Section n. 7. General genetics and genomics

Beyond FD: the bZIP AREB3 mediates FT signalling and floral transition at the Arabidopsis shoot apical meristem

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The floral transition is a critical developmental change in all flowering plants. It occurs at the shoot apical meristem (SAM) in response to external and internal signals, including daylength variations. In Arabidopsis, long-day photoperiods stimulate the production of the florigenic signal FLOWERING LOCUS T (FT) in the leaf vasculature, which is transported to the SAM, where it binds the bZIP transcription factor FD. Together, they cause transcriptional reprogramming at the SAM, leading to the acquisition of floral identity by lateral primordia. Genetic studies reveal that ft and fd mutants are late flowering, with ft mutants showing a more severe phenotype than fd mutants. This suggests that other bZIP transcription factors may play a role in FT signaling at the Arabidopsis SAM.
One such transcription factor is AREB3, which is expressed at the SAM in a pattern that overlaps significantly with FD. AREB3 contributes to FT signaling and acts redundantly with FD. The presence of a conserved carboxy-terminal SAP domain in AREB3 is essential for downstream signaling. Interestingly, AREB3 and FD have both unique and common expression patterns, with FD negatively regulating AREB3 expression levels, forming a compensatory feedback loop. Mutations in another bZIP transcription factor, FDP, exacerbate the late flowering phenotypes of fd areb3 mutants. These findings suggest that multiple florigen-interacting bZIP transcription factors have redundant functions in flowering at the SAM.

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Section n. 7. General genetics and genomics

Looking for smithRNA candidates across Metazoa

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The recent discovery of small mitochondrial highly-transcribed RNAs (smithRNAs), encoded in the mitochondrial genome and acting as nuclear regulators in the Manila clam, opens to new possibilities concerning the interaction between the two genomes. To determine whether this can be regarded as a novel signaling pathway of general interest, representative metazoan species will be investigated through a new pipeline for the identification of candidate smithRNAs.
The pipeline reproduces the original procedure used to identify smithRNAs in the Manila clam, which evaluates a series of genetic attributes such as remapping coverage, 5'/3' conservation, presence of nuclear targets and folding energy of the pre-smithRNA structure. Improvements of this implementation are the possibility to use replicate samples, an improved clustering that does not necessarily favor longer sequences, calculation of free energy in pre-smithRNAs on both directions and the use of an user defined 'stringency'.
As a preliminary investigation, the pipeline was tested on the Mediterranean fruit fly Ceratitis capitata, where it successfully identified 2 candidate smithRNAs. These candidate sequences exhibited relatively high coverage, well-defined 5' ends and a pre-miRNA-like folding structure. Similarity and thermodynamic analyses carried out using multiple software tools consistently identified an association with different nuclear targets at low levels of free energy ($\Delta G < -20$).

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Section n. 7. General genetics and genomics

How to get rid of the Y chromosome from a cage population of Ceratitis capitata: effects on sex ratio during eight generations of XX-only individuals.

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Ceratitis capitata is an agricultural insect pest belonging to the Tephritidae family (Diptera). The presence of the Y chromosome determines its sexual fate: XX embryos develop into females by a female-determining master gene regulator (Cctra), and XY embryos into males by a Y-linked master gene that determines masculinity (MoY) but is not required for fertility. An artificial male determining transgene (T indicates the RNAi-transgene genotype and + its absence) was introduced into the Medfly genome producing Cctra-specific dsRNA during female oogenesis but not in the somatic (see Volpe et al., poster, AGI2023). A population of Medfly lacking the Y chromosome, called MinusY, was established, containing XX males (having three possible TT, T+, and ++ genotypes) and XX females (having two possible T+ and ++ genotypes). 12.000 XX-only individuals were screened over eight generations. A sex ratio in favor of XX females versus XX males was observed (approximately 1.4) and the frequency of the T transgene was approximately 0.3. We developed an Individual-Based Model to compare predictions and observations and to derive the minimum number of flies required for each generation to maintain the stability of the MinusY population.

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Section n. 7. General genetics and genomics

Antarctic microbial genomics: insights into cold adaptation and secondary metabolite biosynthesis

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Antarctica's extreme conditions host diverse microorganisms crucial for ecosystem functioning, but knowledge remains limited. In the research to explore the biological potential of Antarctica's microorganisms, we focused on the genomes of 71 bacterial strains that were isolated from Antarctica and that form part of the Collezione Italiana Batteri Antartici (CIBAN), University of Messina. These genomes were sequenced, assembled and preliminary analysed to unravel the genetic basis of their adaptation to this unique environment. The CIBAN collection, established in 1989, harbors 518 cold-adapted bacterial strains gathered from diverse Antarctic regions, yet comprehensive -omics data have been lacking.
Our genomic analyses include in-depth taxonomic and phylogenetic assessments, enabling studies of comparative genomics. Afterwards, we deciphered the pangenome of each cluster of identified genera, unveiling the distinctive functions inherent in these organisms. In particular, the purpose of these analyses is to identify genes related to cold adaptation and the biosynthesis of secondary metabolites, shedding light on the strategies employed by bacteria to thrive in the extreme conditions of Antarctica.
In conclusion, our comprehensive genomic analysis of 71 Antarctic bacterial genomes will provide crucial insights into their functional repertoire. The genomic data open new horizons for understanding bacterial life in Antarctica and offer practical solutions for preserving this delicate ecosystem.

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Section n. 7. General genetics and genomics

The Ser727 mutated form of mitochondrial Stat3 can affect the cellular response to genotoxic stress

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A coordinated action between nuclear and mitochondrial activities is essential for a proper cellular response to genotoxic stress. Several nuclear transcription factors, including STAT3, translocate to mitochondria to exert mitochondrial function regulation; however, the role of mitochondrial STAT3 (mitoSTAT3) under stressed conditions is still poorly understood. In this study, we examined whether the stable expression of mitoSTAT3 wild-type or mutated at the conserved serine residue (Ser727), which is involved in the mitochondrial function of STAT3, can affect the DNA damage response to UVC radiation. To address this issue, we generated mammalian cells (NIH-3T3 and HCT-116 cells) stably expressing exogenous Stat3 gene with wild-type or mutated Ser-727 residue, with the substitution of the serine to an alanine (S727A, to block Serine phosphorylation) or aspartatic acid (S727D,to mimic the Serine phosphorylation) and with a mitochondrial localization sequence (MLS) and a nuclear export sequence (NES) that makes STAT3 unable to localize into the nucleus. Our results show that cell proliferation is enhanced in mitoStat3-transduced cells under both non-stressed and stressed conditions. Once irradiated with UVC, cells expressing wild-type mitoSTAT3 showed the highest cell survival, which was associated with a significant decrease in cell death. Low levels of oxidative stress were detected in UVC-irradiated NIH-3T3 cells expressing mitoSTAT3 wild-type or serine-related dominant active form (Ser727D), confirming a role of mitochondrial STAT3 in minimizing oxidant cellular stress that provides an advantage for cell survival.

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Section n. 7. General genetics and genomics

World-wide invasion of Popillia japonica reconstructed based on complete mitochondrial genomes and nuclear SNP markers.

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The Japanese beetle Popillia japonica is a pest of significant economic interest as it feeds on hundreds of species of wild and cultivated plants, including important fruit, vegetable and field crops. Native to Japan, the pest has recently invaded large areas of the USA, Canada, the Azores (Portugal), Italy, and Ticino (Switzerland) and is considered a priority for control in the EU.
We used molecular markers and population genomics tools on samples covering the entire distribution of the species to study: a) the geographic differentiation of the species; and b) the process of expansion from its native area to the USA and to Europe. The dataset includes an alignment of 86 complete mitochondrial genomes and a panel of 3'666'428 nuclear SNPs, or 295'396 unlinked SNPs, genotyped by WGS resequencing in 83 individuals.
Main results are: a) the existence of 5-6 genetic groups of P. japonica, grossly corresponding to different areas of colonization at a continental scale, with extensive mixing within areas; b) a primary event of differentiation between South and Central/North Japan, with all invasive samples coming from the latter; c) two separate introductions to the Azores and Italy starting from the USA; d) uniformity of cross border introductions in Canada and Ticino with the neighboring populations in USA and Italy; and e) a generalized loss of diversity associated to invasion events and little evidence for directional selection.

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Section n. 7. General genetics and genomics

Exploring Autophagy's Impact on DNA Repair Pathways: Unraveling Mechanisms and Implications for Cancer Therapies

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It is becoming increasingly clear that, despite being a cytoplasmic process, autophagy plays a key role in maintaining genomic stability. Many studies provided evidence that autophagy may modulates DNA repair pathways, with major implication for therapy-induced responses and acquired resistance in cancer, although the exact mechanism behind this connection remains a matter of debate. To investigate how autophagy modulates DSB damage response, we have generated a cell line expressing the fusion protein consisting of the AsiSI restriction enzyme and a modified hormone-binding domain from the estrogen receptor. Cell exposure to 4-hydroxytamoxifen results in nuclear accumulation of the AsiSI-ER protein and in the rapid induction of ~150 sequence-specific DSBs across the genome. This cellular system enables us to investigate recruitment of DNA repair factors at specific DSBs by using ChIP-based approaches. To investigate how autophagy modulates DSB damage response, we employed CRISPR/Cas9 genome editing to establish a ATG7 KO AsiSI-ER cell line. Our goal is to investigate how autophagy regulates DNA repair proficiency and pathway choice throughout the cell cycle and the molecular events underlying these regulatory mechanisms.

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Section n. 7. General genetics and genomics

Reconstructing the genetic composition of early modern individuals from the crypt of Ca' Granda hospital (Milan, Italy)

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Archaeogenomics represents a precious tool to reconstruct the history of past populations. Up until now, only few publications have included individuals from late medieval and early modern Southern Europe and even less from Italy. In this study, five ancient petrous bones recovered from the crypt of Ospedale Maggio-re (also known as Ca' Granda), one of the main healthcare institutions in Milan (Italy) in the 17th century, were processed to obtain low-coverage whole genomes through sequencing on Illumina platform. Se-quenced reads underwent an extensive validation procedure and, eventually, three whole genomes were eligible for further investigation. This focused on molecular sex determination, phylogenetic and phylogeo-graphic analyses at both uniparental (mitochondrial DNA and Y chromosome) and whole-genome level. In particular, the three Ca' Granda individuals were compared to a genome-wide dataset representative of modern and ancient samples from across Italy, Eurasia and North Africa to determine their biogeographical origin. Overall, the results contributed to reconstruct the biological profile of the individuals and the genetic past of Milan.

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Section n. 7. General genetics and genomics

Human cardiac microtissues capture aberrant RNA splicing in doxorubicin-induced cardiotoxicity

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Doxorubicin (doxo) is a chemotherapeutic drug largely used for its efficacy in killing tumours. However, doxorubicin-induced cardiotoxicity (DIC) is a major side-effect causing morbidity and mortality among oncology patients. Different patterns of RNA splicing regulate the pathophysiology of many organs, especially of brain and heart, and RNA Binding Proteins (RBPs) have proved to be master regulators of RNA splicing during heart development and function. Notably, doxo was shown to alter RBPs expression and this may cause aberrant splicing in the heart.

Human induced Pluripotent Stem Cells (hiPSCs) can be differentiated in 2-dimensional (2D) hiPSC-derived cardiomyocytes (CM) that have been largely used to study DIC in vitro. However, 2D-CM immaturity is a barrier to the recapitulation of post-natal heart disease and remodelling.

Here, we use 3-dimensional hiPSC-derived cardiac microtissues (MTs) to detect DIC. We show that hiPSC-CM maturation in our 3D MT model is regulated by a group of RBPs and that these are sensitive to doxo, thus causing aberrant RNA splicing. Further, we are studying how these splicing events are altered in MTs built with hiPSCs-derived from patients genetically predisposed to DIC.

In summary, we show that 3D hiPSC MTs capture doxo-induced aberrant RNA splicing and therefore represent a useful tool for studying in vitro the molecular mechanisms underlying DIC.

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Section n. 7. General genetics and genomics

Blurry promoters: from genome evolution to biotechnological applications

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“Blurry promoters”, short DNA sequences derived from Class II transposon elements (TE), have the unique ability to activate the transcription of downstream genes in different and unrelated cellular contexts. To date four promoters isolated from transposons belonging Tc1/mariner (REF, cells), PiggyBac and P superfamilies fall within this category. These promoters have been tested in Prokaryotic (E.Coli) and Eukaryotic (yeast, Drosophila and Human) model cellular systems, showing a non-homogeneous transcription efficiency and classifying them as weak promoters.
From an evolutionary perspective, promiscuous promoters may play a pivotal role in facilitating Horizontal Transposon Transfer (HTT) process enabling TEs can disseminate among phylogenetically unrelated species. Furthermore, weak promoters could potentially help evading over expression inhibition of the transposase.
In the field of biotechnology, a broad-spectrum promoters hold great promise for the development of multi-host expression vectors. Employing genetic engineering multi-host tools represents a crucial step towards a cost-effective and time-saving approach to implementing research protocols.
Here we explore the performance of the Bari1 and PiggyBac blurry promoters in different applications, such as the expression of a drug resistance cassette, the Cas9 expression for in vivo and in vitro gene editing, and the transposase expression for in vivo and in vitro transposition assays.

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Section n. 7. General genetics and genomics

Titanium Dioxide Nanoparticles: uptake, cyto- and genotoxic effects on Caco-2 intestinal cells.

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Titanium Dioxide Nanoparticles (nano-TiO2), are widely used in cosmetic industries, for sunscreens and paints, thanks to their whitening power and, more recently, in biomedicine. Moreover, nano-TiO2 is used in food products and additives, leading to an abundant amount ingested by consumers.
Thus, it is necessary to investigate the possible effects of nano-TiO2 on the gastrointestinal tract to evaluate its safety for human health. For this reason, human epithelial colorectal adenocarcinoma cells were selected as an in vitro model.
This study is aimed at determining the uptake capacity and potential genotoxic and ultrastructural effects of nano-TiO2 exposure on Caco-2 cells.
Caco-2 cells were exposed to nano-TiO2 Anatase (20, 50, 100 e 150 µg/ml); cell viability and primary DNA damage were evaluated by Trypan blue and Comet assay, respectively. Ultrastructural analysis was carried out by transmission electron microscopy (TEM) to evaluate potential damages at subcellular level. A statistically significant increase ($p<0.05$) in DNA damage was detected after 4 hours of exposure to 20 and 150 µg/ml of nano-TiO2. TEM analysis showed an increasing presence of nano-TiO2 inside the cells, which was proportional to the exposure dose. The presence of damaged mitochondria was found at any exposure dose. These results showed that nano-TiO2 contained in food products can be internalized and is potentially harmful to the intestinal tract. Careful management of its use regulation is recommended.

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Section n. 7. General genetics and genomics

A genotoxicity approach to study the effectiveness of ecofriendly nanomaterials in remediating freshwater polluted sediments.

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Nanoremediation is an innovative solution for the removal of pollutants from contaminated sites, based on the use of nanoparticles and engineered nanomaterials.
Here we tested the effectiveness of nano-sized ecofriendly cyclodextrins in reducing toxicity of contaminated freshwater sludge in combination with filtration by geotextile fine-mesh net.
Three treatment groups were set-up: artificial freshwater as control; waters obtained by filtering the polluted sludge with or without cyclodextrins.
The effectiveness of remediation was evaluated by in vivo exposure of D. polymorpha and A. fischeri to the resulting waters. Loss of DNA integrity evaluated by Comet assay, chromosomal damage assessed by Cytome assay and the inhibition of bioluminescence were observed. Sediment toxicity was detected by H. incongruens mortality test.
Starting sediment showed very high toxicity while sludge treated combining cyclodextrins and pressing resulted in a reduction of toxicity in H. incongruens. The water obtained by filtering sludge without cyclodextrin treatment was found to exert acute toxicity in A. fischeri and to be cito- and genotoxic for D. polymorpha, showing an increase of DNA damage, nuclear anomalies and apoptotic cells frequency compared to controls. Moreover, toxicity was reduced in specimens exposed to waters obtained by pressing cyclodextrin pre-treated sludge.
Present data support the combined treatment of nanomaterials and filtration as a promising tool in the remediation field.

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Section n. 7. General genetics and genomics

YB-1 oncoprotein as an oxidative stress sensor and DNA damage repair factor

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Y-box binding protein-1 (YB-1) is overexpressed in several tumor types. Enhanced expression of nuclear YB-1 is associated with tumor aggressiveness, drug resistance, and poor prognosis in cancer patients. However, the molecular mechanisms contributing to YB-1 oncogenic functions remain still undefined. YB-1 belongs to the nucleic acid binding cold shock protein superfamily containing the evolutionarily conserved cold shock domain (CSD) that enables it to bind nucleic acids. YB-1 protein functions are strictly dependent on its subcellular localization. YB-1 is a sensor of oxidative stress and a key component of stress granules. However, evidence points towards a relevant role of YB-1 in DNA damage repair. YB-1 is phosphorylated at serine 102 by AKT and S102 phosphorylation was shown to activate and promote YB-1 nuclear import. We have already provided evidence of the essential role played by YB-1 in stress response and cell survival. Here, we present data showing an important role of YB-1 in DNA damage repair. Our data indicate that YB-1 is a target of ATM and stress-activated c-Jun N-terminal Kinase (JNK). We show that the phospho-defective S102A mutant of YB-1 despite its ability to efficiently translocate into the nucleus. It is defective in DNA damage repair upon stress stimuli. Inhibition of the ATM and JNK signaling pathways that regulate YB-1 nuclear translocation could provide novel cancer therapy strategies.

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Section n. 7. General genetics and genomics

Fine-scale characterization of the 15q11-q13 locus in human and primate genomes reveals biomedically relevant hotspots

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The impact of segmental duplications on human evolution and disease is only just starting to unfold, thanks to advancements in sequencing technologies that allow their discovery and precise genotyping. The 15q11-q13 locus is a hotspot of recurrent copy number variation associated with Prader-Willi/Angelman syndromes, developmental delay, autism and epilepsy, and is mediated by complex segmental duplications, many of which arose recently during evolution. To gain insight into the instability of this region we sought to characterize its architecture in human and nonhuman primates, reconstructing the evolutionary history of five different inversions that rearranged the region in different species primarily by accumulation of segmental duplications. Comparative analysis of human and nonhuman primates duplication structures suggest a human-specific gain of directly oriented duplications in the regions flanking GOLGA and HERC cores, representing the starting point of the expansion. The increasing complexity of segmental duplication organization over the course of evolution underlie their association with humans susceptibility to recurrent disease-associated rearrangements.

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Section n. 7. General genetics and genomics

Exposome and genome interactions and pancreatic ductal adenocarcinoma susceptibility in the UK Biobank

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Pancreatic ductal adenocarcinoma (PDAC) arises from lifestyle, environment, and genetic factors. Previous gene-environment interaction studies considered a limited number of polymorphisms and risk factors. Thus, this study aims to analyse the exposome on PDAC susceptibility and identify possible genome-exposome (GxE) interactions, to identify high risk individuals.

The study analysed 373 exposome variables in 816 PDAC cases and 302,644 controls from the UK Biobank cohort. Correlation matrixes were used to identify 358 independent variables ($r2<0.80$). Additionally, a weighted polygenic risk score (PRS) was computed using all susceptibility loci ($P<5x10-8$). The threshold for statistical significance was set at $P<1.40x10-4$ for multiple testing. GxE interactions were calculated using multiplicative models for significant variables and PRS.

A total of 53 associations under the Bonferroni corrected threshold were observed. The PRS showed a significant association, for the highest versus lowest quintile ($P=2.09x10-9$). Among exposome variables, heavy alcohol drinking ($P=3.39x10-7$), smoking ($P=7.31x10-17$), high fat-free mass ($P=2.39x10-22$), sedentary behaviours ($P=5.72x10-4$) and stress-related factors ($P=2.00x10-16$) were significantly associated with PDAC risk. However, no statistical interactions were observed in the GxE analysis.

Our results show very clear associations for the genome and the exposome with PDAC risk. However, there are no interaction between the two.

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Section n. 7. General genetics and genomics

Artificial intelligence approaches to predict pancreatic ductal adenocarcinoma risk

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Introduction: The ability to accurately predict the individual risk to develop pancreatic ductal adenocarcinoma (PDAC) is crucial for the implementation of prevention. Therefore, this study aimed at testing different machine learning (ML) methods to predict the occurrence of PC.
Methods: Using 347 variables (genetic and non-genetic) from the UK Biobank cohort, we developed a predictive model for PDAC with 816 PDAC cases and 302,644 controls. Five ML predictive models (Random Forest, AdaBoost, XGBoost, CatBoost, and DeepForest) were tested with two variable sets (those significant under Bonferroni and $p<0.05$ thresholds). Model evaluation included AUC, accuracy, precision, recall, and F1-score. SHapley Additive exPlanation (SHAP) was applied to the best ML models to identify important features and explain prediction focus.
Results: Among the 5 models tested, CatBoost showed the best predictive performance (AUC=0.92 and recall=0.77) for the first group of variables (n=25). XGBoost performed best (AUC=0.92 and recall=0.75) for the second group of variables (n=56). SHAP explanation highlighted age and polygenic risk score (PRS) as major contributors to XGBoost and CatBoost models' predictive performance.
Conclusion: ML showed high performance to predict PDAC occurrence even with a relatively small number of PDAC cases, increasing the number of patients tested in the model will, however, be instrumental to apply them in a clinical setting.

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Section n. 7. General genetics and genomics

Inference of phenotypic traits from low coverage ancient genomes: an operating manual

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The forensically validated HIrisPlex-S system represents the most popular model for the simultaneous prediction of eye, hair, and skin colours. Based on the analysis of 41 pigmentation gene polymorphisms, it allows the estimation of individual probabilities for three eye, four hair and five skin colour categories by analysing only genotypic data.
This tool was primarily developed to aid specific criminal investigations, in cases with inconclusive STR analysis, but, in the last decade, many studies have aimed to predict the phenotype of ancient human skeletal remains using the well-validated HIrisPlex-S system.
There are several aspects that forensic DNA analysis and studies of ancient DNA (aDNA) have in common, such as DNA degradation that results in loss of data.
The aim of this study is to test the robustness of the HIrisPlex-S system when applied to low coverage data, and to evaluate the power of this phenotypic inferential procedure when dealing with methods that explicitly take into account the uncertainty of the genotype calling in low coverage data, or relying on imputation to estimate genotypes. The final purpose is to provide an operating manual for the inference of phenotypic traits from low coverage and ancient genomes.
Based on our guidelines, we inferred geographical distribution of human eye, hair, and skin pigmentation on 448 Eurasian individuals, spanning from the Upper Paleolithic to the Iron Age.

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Section n. 7. General genetics and genomics

NEWPAT: development of a non-invasive paternity test with high specificity

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The Non-Invasive Prenatal Paternity Test (NIPAT) builds upon the discovery of small amounts of cell-free fetal DNA (cffDNA) circulating in maternal blood. The current non-invasive tests rely on single nucleotide polymorphisms (SNPs), with limitations due to low fetal DNA fractions and high error rate of targeted next-generation sequencing (NGS). To address this, our project aims to exploit a new class of genetic markers, biallelic double nucleotide polymorphisms (DNPs). The probability of being sequenced incorrectly is the product of the individual base sequencing error probabilities, reducing the NGS error rate by two orders of magnitude. First, a bioinformatic approach has been used to identify 978 genome-wide DNPs from the large amount of whole genome sequences in the Genome Aggregation Database. These markers were analyzed in 15 individuals from 4 families, amplifying their DNA using Ion AmpliSeq™ Kit Plus from Thermo Fisher Scientific. Different depths of coverage were explored for the same samples, reaching a maximum of 5000x, while 6 full-risk samples with varying fetal fractions were tested to validate the minimum detectable and informative quantity. The NGS amplification quality of the selected DNPs in all the samples has been evaluated in different steps, by performing a depth analysis and by calling all the alleles at each position in order to estimate the background noise and distinguish the false calls due to sequencing errors from the true cffDNA alleles.

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Section n. 7. General genetics and genomics

Unraveling the Interplay of 8-oxodG and G4: Implications for DNA Damage and Transcription Regulation.

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Reactive oxygen species can induce guanine oxidation, leading to the formation of 8-oxo-7,8-dihydroguanine (8-oxodG), a common DNA base lesion. The base excision repair (BER) pathway is responsible for repairing 8-oxodG, but incomplete repair may result in mutagenesis and genome instability. Notably, 8-oxodG plays a crucial role in epigenetic gene expression control through its interactions with G-quadruplex (G4) structures. Regions containing putative G4 forming sequences (PQS) are prone to 8-oxodG formation, thereby affecting the stability of G4 structures and subsequently influencing gene expression. Pharmacological and/or genetic targeting of BER proteins offers a promising approach to manipulate the levels of 8-oxodG and G4. In our laboratory, we have established both normal and cancerous BER-deficient human mammary epithelial cell lines. Immunofluorescence assay highlighted an increased number of y-H2AX foci in BER protein-deficient cells. These data suggest that BER deficiency results in the accumulation of endogenous DNA damage and in the formation of double-strand breaks (DSBs). This may promote genome instability. However, further experiments are needed to confirm these results.
Finally, BER-deficient cell lines will be useful in future to investigate the crosstalk between 8-oxodG and G4 and identify transcriptional alterations in human diseases such as cancer.

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Section n. 7. General genetics and genomics

Evolution of quorum sensing regulation through the acquisition of additional feedback loops

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Bacterial quorum sensing (QS) is a cell-to-cell communication systesem in which specific signals are activated to coordinate, for example, pathogenic behaviors and help bacteria collectively respond to perturbations. QS in Gram-negative bacteria is typically regulated by a N-acyl-homoserine lactone (AHL) molecules-mediated system, homologous of Vibrio fischeri LuxI-R. In many cases, bacteria possess more than one QS system, based on different types of molecules, that interact through a complex regulatory network. Presumably, these configurations have emerged over time from simpler ones through the acquisition of novel players (e.g. transcription factors) that have been successfully integrated into the native regulatory systems. However, the advantages provided by these alternative/additional configurations on QS-related phenotypes is poorly predictable only based on their underlying network structure.
Here we studied to which extent horizontal gene transfer has contributed to the extant distribution of LuxIR-like quorum sensing modules in prokaryotic genomes. Using machine learning and genomic composition analysis we classified LuxI- and LuxR-like sequences of 32,482 prokaryotes into native and non-native (i.e. likely acquired through Horizontal Gene Transfer, HGT) and integrated this information on the corresponding phylogenetic tree. Our classification reveals a dynamic gene gain/loss distribution of LuxIR-like systems across at least 70 species of different phyla. Next, we focused on one specific case, i.e. the well-known cci genomic island previously characterised in the Burkholderia genus and known to harbour (among other genes) an "extra" QS regulation module. We investigated the effects of this additional regulation over the native QS system by generating mutants harbouring a reduced (core) regulatory circuit and compared their QS response with the wild type circuit (complete). Experimental results indicate that one of the effects of an additional QS regulation module resides in its capability to buffer the variability of final cell densities in growing populations of Burkholderia cells, thus probably reducing cell-to-cell variation of growth phenotypes. Finally, we implemented a mathematical model that reproduced the experimental observations and that allowed the investigation other possible consequences of this horizontally transferred QS module. Not only do we propose a scenario in which the additional feedback loops are acquired horizontally, but we also speculate that the original CepIR system might have been transferred multiple times across several bacterial families, thus expanding our understanding of the effects of acquired DNA on existing molecular circuitries. In conclusion, our results illuminate on the possible, non-trivial, phenotypes that may arise because of HGT events.

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Section n. 7. General genetics and genomics

Evolution of the Y-linked MoY male determining factor in Ceratitis and Batrocera genera: structural and functional predictions

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The Y-linked MoY gene is sufficient and necessary for Ceratitis capitata male sex determination, as previously shown by eRNAi, CRISPR/Cas9 and transient gain-of-function experiments (Meccariello et al., 2019). MOY is able to promote either directly or indirectly male-specific non-productive splicing of the female-determining Cctransformer pre-mRNA. MoY orthologues are necessary for male sex determination also in the olive fly (B. oleae) and the oriental fruit fly (B. dorsalis) and shown to be conserved in few other Bactrocera species. The MOY protein is very short (70 aa) and lacks similarity to any protein domains, leaving open the questions concerning its structure and function. Preliminary attempts to gain insights into the structural properties of MOY proteins by using predictive approaches based in machine-learning techniques and a limited number of sequences did not provide convincing results. We have searched and found in available databases from other Tephritidae species 12 novel MOY orthologues belonging mostly to Ceratitis genus. Their sequence comparative analyses are consistent with a phylogenetic tree derived from genome comparisons. We are currently performing predictive analyses exploiting the extended database of MOY sequences here described. The results of these investigations and their implications for the understanding of MOY function(s) will be illustrated.

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Section n. 7. General genetics and genomics

Testing different de novo assembly pipelines using simulated ancient metagenomic data

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In the last years, the interest in ancient metagenomics, the analysis of complex DNA content recovered from biological material, is increasing with significant advancements in paleogenomic technologies resulting in the characterization of over 1,700 ancient microbial genomes, partial genomes, and metagenomes. However, to date, most studies focused on aligning sequences to modern reference genomes, limiting discoveries to known taxa and their close relatives, and introducing a reference bias, since the genetic variability present in the modern genome is different from the one present in the ancient genome. A way to overcome this issue is performing a de novo assembly of metagenomes, resulting in a set of genomes of all the species that are present in the sample. However, this result is difficult to achieve since it is recent and it needs high-quality genomic data consisting of long contiguous sequences, which can be challenging to assemble from fragmented ancient DNA (aDNA) using current methods. Several pipelines have been created and applied in different studies, however, to date, there is not a golden standard to follow when analyzing ancient metagenomic data.
In this study, we tested different de novo assembly pipelines in both simulated aDNA and ancient dental calculus. We compared the level of completeness and contamination of the different generated assemblies to identify the pipeline that can better reconstruct the ancient bacterial genome.

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Section n. 7. General genetics and genomics

Mesothelin-binding Fn3 as a novel therapeutic tool for mesothelioma

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Malignant mesothelioma (MM) is a fatal cancer lacking effective therapeutic strategies. Mesothelin (MSLN) is considered a promising therapeutic target, being overexpressed on the surface of MM cells. Antibody-drug conjugates targeting MSLN have been developed, but their therapeutic efficacy remains limited due to their large size (~140 kDa). Smaller scaffolds (5-30 kDa) have recently been proposed to overcome this limitation. Here, we evaluated a small scaffold derived from the tenth domain of type III of human fibronectin (Fn3, ~11 kDa) for its ability to bind MSLN. Firstly, we generated two MSLN-overexpressing cell lines starting from the MM cell lines MSTO-211H. Then we evaluated the Fn3-MSLN binding affinity through flow cytometry and immunofluorescence assays. Flow cytometry indicated that this variant of Fn3 (named Fn3_5.3.2) bound MSLN with high affinity (~11nM). Immunofluorescence confirmed this binding and highlighted the cell surface localization of the Fn3-MSLN complexes. To assess whether the conjugation of Fn3 with chelator molecules, crucial for its radiolabelling, could hamper its affinity for MSLN, we carried out similar evaluations using a DOTA-GA-conjugated Fn3. The preliminary results suggest that the conjugation process has only a limited effect on the Fn3-MSLN binding.
Overall, our work revealed that Fn3_5.3.2 has a high affinity and specificity for MSLN and could be used to deliver cytotoxic molecules for treating MM and other MSLN-overexpressing cancers.

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Section n. 7. General genetics and genomics

Vitellogenin receptors and the low-density lipoprotein receptors family in basal Sarcopterygii

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Proteins belonging to the low-density lipoprotein receptor (LDLRs) superfamily, cell surface receptors, are important for the transport of different ligands across cell membranes. The LDLRs gene family includes 15 receptors such as very low-density lipoprotein receptors (VLDLR), LDLR, Sorting-related receptor with A-type repeats (SORLA) and 12 LDL receptor-related proteins (LRPs). Most of these are not only involved in endocytosis but also in the transduction of important signals during embryonic development and in the regulation of cholesterol homeostasis. All members of this family have three domains: one cytoplasmic, one transmembrane, and one extracellular. In oviparous animals, the VLDL-receptor is also known as VtgR because it facilitates the uptake of vitellogenin, and the same function seems to be played by other members of this family. In tetrapods, information concerning vitellogenin receptors and the LDLR superfamily is limited to few taxa. To increase knowledge on the evolutionary history of this gene family in vertebrates, we focused on basal sarcopterygians, organisms occupying a key phylogenetic position, such as Latimeria chalumnae and Protopterus annectens. Moreover, we also considered Cynops orientalis and Pleurodeles waltl as amphibian species evolved in Devonian and fundamental in the mediation of the transition from water-to-land during tetrapod evolution.

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Section n. 7. General genetics and genomics

A systems biology-oriented investigation of Arabidopsis retrograde-signaling-defective mutant gun1 reveals the co-expression-based HUBs at the center of plastid development and homeostasis

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Although nuclear, plastidial and mitochondrial genomes are physically apart, their gene expression is finely tuned and coordinated. This precise orchestration requires intracellular communication pathways, whose rely on transcriptional, post-transcriptional, translational and post-translational events at whole cell level. The Arabidopsis thaliana GENOMES UNCOUPLED 1 factor has been identified as main player in plastid-to-nucleus retrograde communication, needed for correct plant development and effective adaptation to environmental conditions. In this study, we attempted a holistic description of cellular responses to Lincomycin, a chemical that impair plastid translation and chloroplast development, in Arabidopsis wild-type genetic background and in the retrograde signaling-defective mutant gun1. Our data support the involvement of different cell compartments in the adaptive responses to plastid translation inhibition and identify the most relevant gene networks in each genotype and condition at system level. Specifically, our findings indicate that, in the presence of Lincomycin, cell adaptive responses rely on the plastid compartment in a GUN1-dependent manner, while the lack of GUN1 in the gun1 mutant background activates a set of extra-plastidial molecular networks that lead to chloroplast dismantling, via plastid vesiculation in a PsbO-dependent manner.

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Section n. 7. General genetics and genomics

The dawn of Middle Age: a comprehensive archaeogenetic analysis of the late antiquity site of Forum Sempronii in Central Italy.

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The 5th and 6th centuries CE were characterized by major social and demographic changes in the Italian peninsula. On one hand, the shift in the political balance towards the east from the later Imperial period onwards led to a greater genetic influx from the Near East in the city of Rome. On the other hand, people from central Europe, like Goths, arrived in the Italian peninsula to form their own kingdoms. In addition, the Italian population was also affected by the Justinianic plague, caused by Yersinia pestis, in the VI century. In order to understand how these events affected the Italian peninsula outside the city of Rome, we analyzed the funerary area of the Roman city of Forum Sempronii (V-VI cc. CE, Marche, Italy), as a case-study. This site was first associated with Goths and is characterized by burials that gradually become hastier, possibly suggesting a pandemic event. In this study we performed a comprehensive archaeogenetic analysis on 21 individuals buried in Forum Sempronii. The genetic ancestry of these individuals is in line with samples from the same period from the city of Rome, suggesting that the Near Eastern influx was not restricted to the capital city and possibly rejecting the hypothesis of a non-local origin for this population. Moreover, by analyzing these samples we identified traces of the presence of Yersinia pestis, possibly confirming the hypothesis of the First Pandemic affecting the people in Forum Sempronii.

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Section n. 7. General genetics and genomics

The Picenes and the Genetic Landscape of Central Adriatic Italy in the Iron Age.

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The Italic Iron Age (IA, approx. X-III century BCE) was characterized by the presence of different cultural groups thoroughly studied from an archaeological perspective. Although many genetic studies have been performed, we still miss a comprehensive description of the genetic pool of the ethnicities that lived along the Italian mid-Adriatic coast. To fill this gap, we focused our attention on the Picenes, a civilization that thrived on the Adriatic coasts of Central Italy from the IX century BCE until the Roman colonization. We performed whole genome sequencing in 81 ancient individuals buried in three different IA necropolises located in Central Italy, two belonging to the Picene culture (Novilara and Sirolo-Numana, VIII-VII century BCE) and one Etruscan necropolis (Colle Val D'Elsa, VIII-VI century BCE). Our analysis reveals no major differences between the Picenes and other contemporary populations like the Etruscans, indicating a common genetic origin for the Central Italian IA ethnic groups. Nevertheless, in the Picenes we detected genetic influences from the Balkans and Northern Europe. These findings suggest genetic contacts across the Adriatic Sea and point out the role of the Apennine mountains in partially acting as a geographic barrier to gene flow. Moreover, we identified individuals that show a different genetic origin but that were buried within the Picenes, Suggesting the existence of a multicultural society composed of people from different parts of Europe.

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Section n. 7. General genetics and genomics

Sabethes cyaneus as a model for the study of the genetic control of courtship behavior in mosquitoes

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Insect courtship is a fascinating phenomenon, showcasing a wide range of complex strategies and intricate behaviors employed to ensure mating. Drosophila melanogaster represents the insect model system for studying the genetic basis of mating behavior. In Drosophila the fruitless gene promotes neural pathways necessary for producing courtship songs and executing appropriate courtship behaviors. In contrast, in most mosquito species, like Anopheles spp. and Aedes spp., mating is fast and random, with swarms of males circling and waiting for the sound of female wing beats. Sabethes cyaneus is an extraordinary mosquito species showing a unique and elaborate series of stereotypical mating behaviors and mating on solid surfaces, typically tree sticks. This makes S. cyaneus an ideal candidate to study the role of fru in the regulation of courtship. We analyzed the transcriptome of adults male and female and identified, by in silico approach, the putative fru gene of S. cyaneus. Then, we designed a specific pair of primers to analyze the fru gene amplification by RT-PCR. Our findings revealed the presence of two distinct fru transcript variants, a shorter version in males and a longer version in females. This investigation highlights the putative conservation of alternative sex-specific splicing of the fru gene of S. cyaneus, thereby providing also an opportunity to explore the functional conservation of fru in courtship regulation in mosquitoes.

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Section n. 7. General genetics and genomics

Production of CHM R293X/R293X cell model system to study the rescue of the Rab Escort Protein-1 expression by TRIDs nonsense suppression activity.

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Choroideremia is an inherited genetic disorder caused by several mutations in the CHM gene, which codifies for Rep1 protein strictly linked in intravesicular trafficking. The Rep1 lack causes choroid and photoreceptors degeneration, leading firstly to night blindness and at last to complete blindness. About 39% of mutations on the CHM gene are represented by nonsense mutations, which insert a premature termination codon in the reading frame of respective mRNA, with the production of truncated non-functional protein. Nowadays there is no cure for diseases caused by nonsense mutations, but a promising approach is the suppression therapy using TRIDs molecules (translational readthrough-inducing drugs). In our study, a CHMR293X/R293X cell model system was produced to evaluate the activity of three new optimized molecules (NV848, NV914, and NV930) in the rescue of the Rab-Escort-Protein-1 (Rep1) expression.

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Section n. 7. General genetics and genomics

Selective targeting of NRAS mutations using oligonucleotides and CRISPR system in multiple myeloma

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Multiple myeloma (MM) is a significant hematological malignancy, characterized by approximately 0.9% of newly diagnosed cancer cases worldwide in 2020. Nowadays, the survival of MM patients has significantly increased in the last 15 years thanks to the major understanding of the molecular mechanisms of this disease and to the improvement of the diagnostic and therapeutic strategies. Much still needs to be discovered.

Mutations in oncogenes, particularly in RAS family genes, play a pivotal role in driving the development of various tumors. RAS proteins, acting as small GTPases, activate downstream pathways like MAPK and PI3K/AKT, which promote cell proliferation and survival. The most common point mutations are typically found in codons 12, 13, or 61 of RAS genes. Among the NRAS point mutations, driver mutations Q61R/K stand out as the most frequent across all tumor cases. These mutations lead to the constitutive activation of the proteins, resulting in tumor progression, relapse, and drug resistance.

As NRAS Q61R/K mutations are frequently observed in MM where their precise role has not been fully established. Therefore, we aim to investigate their significance in MM by specifically targeting the mutant allele using CRISPR tools and oligonucleotides (such as siRNA and GapmeRs). By doing so, we intend to examine the cellular effects resulting from the knock-down of the specific allele, leading to a deeper understanding of the implications of these mutations in the context of MM.

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Section n. 7. General genetics and genomics

Unravelling the role of the tumor suppressor p14ARF in mechanotrasduction in tumor cells

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The tumor suppressor p14ARF, encoded by the CDKN2a/ARF locus, halts cell growth by both p53-dependent and independent pathways and is involved in several cellular pathways. Quite unexpectedly, in the last years, a pro-oncogenic ARF function has been observed. In particular, ARF is involved in cytoskeletal remodeling and anoikis protection. However, the molecular mechanisms and the functional implication of these ARF functions remain unknown. We now present preliminary experiments showing that p14ARF downregulation also affects nuclear morphology and is associated with increased nucleus area in HeLa cells. Lamin B protein level, a structural component of the nucleus, also increases in ARF-depleted cells. Besides being required for nuclear envelope integrity, the nuclear lamina, in association with several nuclear components, responds to mechanical forces and is involved in cell mechanotransduction, the pathway that allows cells to transform mechanical signals into biochemical signals and downstream cellular responses. We thus also analyzed protein levels and the subcellular localization of the YAP/TAZ complex, the master effectors of cellular mechanosignalling. As for lamin B, both proteins' levels are deeply affected by ARF depletion. Collectively, our data show for the first time a functional connection between the tumour suppressor ARF and proteins related to mechanosensing, prompting us further to investigate this aspect and its relation to cancer development.

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Section n. 7. General genetics and genomics

Robust demographic inference from low-coverage whole-genome data through Approximate Bayesian Computation

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The reconstruction of past demographic history relies on the pattern of genetic variation contained in the genomes of the sampled populations. Many inferential methods accomplish this task under the implicit assumption that the genotypes at polymorphic sites in genomes are typed without uncertainty. This assumption is addressed increasing the sequencing coverage over 20-30x. Although this approach is cost-effective and provide an accurate characterization of genotypes, it is limited to samples containing high-quality DNA. The sequencing of low-quality samples, like ancient specimens, often results in low-coverage genomes in which the genotype calling is error prone, making such data unsuitable for many inferential methods.
Here, we present a new ABC framework, based on the Random-Forest (ABC-RF) machine-learning approach, to perform demographic inference using low-coverage genomes. Simulated and observed data are compared using genotype likelihoods instead of genotypes to account for the uncertainty that characterize low-coverage samples.
We assessed the inferential power of this framework in distinguishing among different demographic models and in inferring model parameters under different coverage levels, number of individuals, number and size of the genomic loci considered.
Our results showed that the proposed ABC-RF framework provides reliable inferences of the past demographic history paving the way for explicit model comparison through ABC exploiting low-coverage data.

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Section n. 7. General genetics and genomics

Masculinization of XX individuals in the Mediterranean fruitfly Ceratitis capitata by a transgene-mediated maternal RNAi targeting the female determining Cctra

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The Mediterranean fruit fly Ceratitis capitata transformer (Cctra) is a master female-determining gene widely conserved in other agricultural insect pests. We developed a transgene-mediated Cctra-RNAi (T) leading to fully masculinized and fertile XX individuals. We have serendipitously found a maternal effect of transgene integration. The genomic region flanking the integration point contains a hoppel-like transposon, likely conferring a higher transgene expression in the ovaries of transgenic T+ females . The Cctra-specific dsRNA, is deposited from these mothers, also into the non-transgenic X eggs, and represses Cctra, leading to non-transgenic XX male adults. Our data paves the way towards the possibility of sorting male-only non-GM insects from a GM strain. Furthermore, we provide evidence of a molecular mechanism for developing a strategy in which maternal RNAi coupled with conditional expression can lead to a unique strain able to produce on-demand male-only progeny by sexual reversion rather than the available female-specific lethality or male sorting. These masculinization strategies are valuable for different innovative genetic pest control strategies, such as the Sterile Insect Technique (SIT) and the gene drive.

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Section n. 7. General genetics and genomics

The possible recruitment of DROOPING LEAF-like gene DL2 in the molecular pathway at the base of the orchid perianth differentiation

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The Orchidaceae family represents a unique ecological and genetic source for the extraordinary diversification of flower architecture, specialized developmental programs, and pollination syndromes. A complex molecular network underpins orchid perianth organs specification, involving many transcription factors belonging to different families (e.g., MADS, TCP, MYB). In Phalaenopsis orchid, we observed an expansion of a YABBY DROOPING LEAF-like gene expression domain to the perianth. In contrast, in other angiosperms, it is generally involved in reproductive organs and leaf development. In Phalaenopsis, PeDL2 is differentially expressed between the lip and lateral inner tepals, suggesting a neofunctionalization of this gene. To support this hypothesis, we evaluated the expression of the DL-like genes outside the Phalaenopsis genus (Vanilla, Phragmipedium, Rhyncholaeliocattleya, and Dendrobium) by qPCR experiments and in silico differential expression analysis. DL2 possibly acquired a new function in the perianth specification in most ancestral orchids, and later it assumed a specific role in the lip in most recent orchids. To characterize PeDL2, we verified its nuclear localization and predicted its protein structure and interactions by in silico and in vivo approaches. In addition, we analyzed the PeDL2 transcriptional regulation identifying possible promoter interactors.

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Section n. 7. General genetics and genomics

Looking for a mitochondrial role of telomerase reverse transcriptase

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Telomerase Reverse Transcriptase (TERT), aside from the well-established role in telomere lengthening, has non-canonical functions such as gene regulation and protection against apoptosis. In a previous work, we have demonstrated the presence of TERT inside mitochondria in TERT transfected fibroblasts, showing a role in preserving mitochondrial health and functionality. To unravel the putative mechanism of action inside the mitochondrion, we transfected normal primary fibroblasts HFFF2 and two osteosarcoma ALT cancer cells (U2OS and SaOS-2) with HA-tagged TERT protein, obtaining TERT overexpressing cells (HF-TERT-HA, U2OS-TERT-HA and SaOS-TERT-HA). Together with these cell lines, normally lacking TERT expression, we used two telomerase cancer cell lines (HCT-116 and SKMEL-28) which display endogenous TERT. We showed clearly and unambiguously the presence of TERT inside mitochondria in all the cell lines utilized, taking advantage of different techniques (Western blot, co-immunofluorescence and electron microscopy). Since it is known that in the nucleus TERT is involved in mechanisms other than telomere maintenance, such as gene expression regulation of WNT/β-catenin or NF-kB pathways, we hypothesized that in the mitochondria it could have a similar function. To test this hypothesis, we have performed mitochondrial chromatin immunoprecipitation in order to investigate the possible binding of TERT to specific mtDNA sequences.

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Section n. 7. General genetics and genomics

An overview of Intevine: omics data integration in grapevine

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Intevine shares the vision of the one health approach: living beings are not closed compartments, but are influenced by, and influence, other living beings and the environment. By using of omics data integration we aim at enabling a better understanding of complex interactions.
Grapevine cuttings were obtained from Vivai Cooperativi Rauscedo. Half of them were provided untreated, and half underwent a phyto-sanitary treatment. Cuttings were planted in peat, peat+farmyard manure, or sand. For each soil, 50% of samples underwent autoclave sterilization, and 50% were used as is.
The following data were collected:

1. multispectral images
2. ionomics analysis of leaves
3. chemical and physical properties of the soil
4. Real time concentration of cations in xylem sap (bioristor)
5. mRNA-seq of roots
6. 16s sequencing
7. ITS sequencing

Soil substantially affected microbial composition and the transcriptomic pattern of the roots. No strong effect was caused by soil sterilization and hot phytosanitary treatment of roots.
The relative abundance of bacteria correlated with soil pH, dissolved organic carbon (DOC), dissolved nitrogen (DN), and ATP. Some genera, were negatively correlated with pH and positively to DOC, DN and ATP; other genera were positively correlated with pH and negatively to DOC, DN, and ATP.

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