POSTER SESSION 1
APRIL 08
FROM 16:25 TO 17:25

1

NON-CODING RNA GENES

SSTR5-AS1 AND SEMA3B-AS1 AS POTENTIAL MOLECULAR BIOMARKERS OF OVARIAN CANCER

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Ovarian cancer is a multifactor disease with abnormal molecular changes at the genetic and epigenetic level. The high incidence of adverse outcomes associated with the heterogeneity of tumor types and asymptomatic progression requires the search for new effective molecular markers for timely diagnosis of the disease. Long noncoding RNAs (lncRNAs) play a key role in cancer biology, participating in various cellular processes of carcinogenesis and metastasis. This study is aimed at elucidating the role of lncRNAs in the regulation of tumorigenesis in ovarian cancer.

The purpose of our study is to evaluate the effect of DNA methylation on changes in the expression level of the lncRNA genes SSTR5-AS1 and SEMA3B-AS1 in ovarian cancer.

Methylation and expression levels were analyzed using MS-qPCR and RT-qPCR, respectively on the set of 27 samples. The nonparametric Mann-Whitney and Spearman criteria for independent events (RStudio) were used to assess the significance of differences between the study groups ($p \leq 0.05$).

A statistically significant increase of the methylation level in tumor samples was shown for the SSTR5-AS1 and SEMA3B-AS1 genes (p0.001, FDR=0.1) when compare with the paired norm. At the same time, a decrease level in the expression of these lncRNAs in tumor tissue (p0.03, FDR=0.1) was shown. A negative correlation between changes in methylation and expression levels (rs=(-0.62)-(-0.55), $p \leq 0.003$) was revealed. Moreover, we found a positive correlation between SSTR5-AS1 and SEMA3B-AS1 in methylation level (rs =0.22, p0.01) and in expression level (rs=0.73, p0.0001), which suggests their participation in the regulation of common protein-coding genes.

Thus, the lncRNA genes SSTR5-AS1 and SEMA3B-AS1 are involved in the pathogenesis of ovarian cancer, which allows the use of these lncRNAs as potential prognostic markers. Further validation and investigation of lncRNAs associated with ovarian cancer is needed.

The work was supported by the RSF grant No. 20-15-00368-P.

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RARE DISEASES

THE HUMAN GENOME ORGANISATION (HUGO) EDUCATION COMMITTEE: FACILITATING WORLDWIDE ACCESS TO GENOMIC EDUCATION RESOURCES, TRAINING AND COURSES.

Prof. Edward Tobias[1,2], Dhavendra Kumar[3], Angela Solano[4], on behalf of the HUGO International Education Comm (SCs)[5]

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INTRODUCTION:
The Human Genome Organisation (HUGO) Education Committee, which was established in 2021, now has over 30 international members. They are each active members of other national and international genetic and genomic organisations, including: the European, African, Australasian, Asia Pacific and American Societies of Human Genetics; the European Certificate in Medical Genetics and Genomics (ECMGG) exam committee; Human, Heredity and Health in Africa (H3Africa); and the Genomic Medicine Foundation UK.

OBJECTIVES:
The committee aims to facilitate and enhance cutting-edge human genomic education and training, worldwide, including in low- and middle-income countries (LMIC).

METHODOLOGY:
The HUGO education committee now comprises six active sub-committees, covering: genome sequencing and technology; variant interpretation and genome databases; computational genomics and bioinformatics; clinical genomics and genomic medicine; genetic and genomic counselling; and genomic education for the general public.

RESULTS:
We provide an updated report on, and web-links (with QR codes) to, a wide range of recent activities undertaken by the HUGO Education Committee. These activities include custom-designed HUGO educational web pages that have been developed, facilitating access to numerous worldwide resources, courses and organisations. These pages have now been accessed from 98 countries worldwide, particularly the US, UK, India, Brazil, Italy, Germany, Vietnam, Malaysia, Egypt, Australia and Spain. Further work of the committee currently includes: organising and delivering a Genomic Education Workshop at HGM2024 on multi-disciplinary genomic healthcare, including case presentations; distributing and running an international survey to identify the genomic education needs of non-genetics health professionals globally, with over 300 responses to date; the development of an international curriculum for training in genetic counselling; the creation of smartphone apps and a catalogue of genomics training modules for worldwide education; running the popular Variant Effect Prediction Training Course (VEPTC) and Human Genome Variation Society (HGVS) nomenclature training; and the development of links with other genetic and genomic organisations worldwide.

CONCLUSIONS:
Several activities aiming to facilitate global genomics education have already been completed by the HUGO Education Committee, including the extended presentation by the committee's members at this meeting, and many more activities are underway.

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RARE DISEASES

Prenatal diagnosis by trio clinical exome sequencing: single center experience

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Abstract: Introduction: Congenital anomalies, characterized by structural or functional abnormalities occurring during intrauterine life, pose a significant medical challenge. The World Health Organization (WHO) defines these anomalies, which can manifest prenatally, at birth, or postnatally. Their prevalence is notable, affecting approximately 2–3% of live births and 20% of spontaneously aborted fetuses. This study aims to identify the genetic cause of ultrasound anomalies by clinical exome sequencing (CES) analysis. The focus is on utilizing CES analysis in a Trio setting, involving the fetus and both parents.

Methods: To achieve this objective, prenatal Trio clinical exome sequencing was conducted in 78 fetuses exhibiting ultrasound anomalies or elevated nuchal translucency (NT ≥ 3 mm) with previously negative results from chromosomal microarray (CMA) analysis.

Results: The study revealed pathogenic or likely pathogenic variants in 24% of the analyzed cases (19 out of 78). Noteworthy findings include de novo variants in 42% of cases and the transmission of causative variants from asymptomatic parents in 58% of cases. The overall diagnostic yield reached 24% even after obtaining negative results from chromosomal microarray analysis.

Conclusion: Trio clinical exome sequencing stands out as a crucial tool in advancing prenatal diagnostics, surpassing the effectiveness of relying solely on chromosomal microarray analysis. This underscores its potential to become a routine diagnostic standard in prenatal care, particularly for cases involving ultrasound anomalies.

ETHICAL, LEGAL AND SOCIAL ISSUES ON HUMAN GENETICS

PRECISION P4 MEDICINE, ITS ASSOCIATED LEGAL CHALLENGES AND THE RELEVANT SOUTH AFRICAN REGULATORY FRAMEWORK

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Precision medicine is a subset of the greater P4 medicine approach. P4 is the acronym used to describe medicine that is preventative, predicative, personalised and participatory. A P4 approach with its emphasis on tailored medical treatment may be regarded as beneficial to health and genomic research in that it offers improved disease prevention, patient engagement and empowerment, enhanced public health outcomes, genomic advancement and precision treatment, and opportunities for research collaboration and innovation. However, following this approach to medicine is not without its complications and it presents certain legal challenges.

South Africa has a high burden of disease and could benefit enormously from precision medicine and a P4 approach. South Africa also holds great potential as an ideal setting for conducting genomic health research as the country hosts a large genetically diverse population which also has unique genetic factors, has a history of strong infectious disease and pharmacogenomic research along with varied health challenges, it has capacity building initiatives and robust ethical and regulatory frameworks and it has a collaborative research environment.

For South Africa's potential as ideal research site to be realised, however, researchers, both domestic and foreign, must be knowledgeable and appreciative of the legal framework whereby health research is regulated. With reference to P4 medicine, the components of prediction, personalisation and participation entail challenges related to privacy, consent and genetic non-discrimination. It is therefore the aim of this paper to elucidate the legal framework surrounding these issues so as to create awareness among researchers in genomic health research in order to improve and encourage scientific development and utility in South Africa.

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MICROBIOME AND METAGENOMICS

MICROBIOTA FROM ORAL RINSES OF ORAL AND OROPHARYNGEAL CANCER PATIENTS

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The analysis of the oral microbiota associated with oral and oropharyngeal squamous cell carcinoma has been limited. This study aimed to evaluate differences in the microbiota of oral rinse samples from patients with oral and oropharyngeal squamous cell carcinoma compared to non-cancer, normal patients. Oral rinse samples were gathered from 20 normal individuals and 40 cancer patients. The alpha and beta diversity of the microbiota samples were examined using OTUs clustered based on 16S rRNA gene sequences acquired through the 16S MetaVxTM method, including MetaVxTM library preparation and Illumina MiSeq sequencing. Data analysis was conducted using the QIIME data analysis package, and Picrust2 (2.3.0) was utilized to predict functional metabolic pathways based on marker gene sequences. Distinct collections were observed at the OTU level in cancer patients compared to normal samples. Based on the abundance of microbial genera, the Linear Discriminant Analysis Effect Size (LEfSe) was used to differentiate the microbial signature at the genus level. We identified significant shifts and increased dysbiosis associated with cancer patients. The prevalence of the genus Prevotella was ascribed to cancer, while the dominance of the genera Streptococcus and Haemophilus was attributed to non-cancer controls. The PICRUSt predictive metagenomic analysis revealed notable differences in KEGG pathways, particularly in histidine metabolism, streptomycin biosynthesis, and valine, leucine and isoleucine biosynthesis. These findings may provide insights into understanding the potential role of oral microbiota in the cancer environment by affecting bacterial abundance, the bacterial ecosystem, and their metabolic function. Further research is necessary to determine the biological significance of these alterations, validate metabolic shifts, and explore bacterial interrelationships in the oral microbiome of cancer patients.

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GENETICS OF COMPLEX DISEASES

GENOMIC ANALYSIS OF AN EGYPTIAN FAMILY WITH HEREDITARY PERIPHERAL NEUROPATHY DUE TO SORD GENE MUTATION

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Background

Sorbitol dehydrogenase (SORD) Deficiency, or SORD-CMT2, is a recently discovered type of axonal Charcot-Marie-Tooth (CMT2) & distal hereditary motor neuropathy (dHMN). SORD-CMT2 is an autosomal recessive hereditary peripheral neuropathy (HPN). We present the genotype-phenotype correlations in a big Egyptian family with 14 diseased members having CMT2 caused by SORD gene mutation.

Patients/methods: All diseased family members have autosomal recessive (AR) pattern of inheritance. Whole exome sequencing (WES) was performed in 3 probands (a father and 2 sons) and a healthy son (in 2017) after extraction of genomic DNA from blood samples. Mutations were confirmed by segregation of the disease in the family & validated by Sanger sequencing. A detailed genotype-phenotype analysis was performed in 12 diseased members (a father and 2 sons (published: Cortese et al. Nature Genetics 2020) and (2 sons, a sister and 6 grandchildren (2022) (unpublished).

Results: This family has 14 members with HPN. They presented with CMT2 phenotype at variable ages of onset. Patients had pathogenic homozygous SORD variant [c.757delG (p.Ala253GlnfsTer27)]. Healthy sons carried the heterozygous variant. All patients presented with distal weakness & wasting of lower limbs, hypoesthesia of stocking distribution, foot drop & deformities & axonal peripheral neuropathy. Other manifestations in different diseased members included progressive sensorineural hearing loss (SNHL), anosmia, autonomic neuropathy and diabetes. This phenotypic spectrum has not been previously reported in published families with SORD-CMT2. The pathogenic mutation is predicted to result in shift of the open reading frame with premature translation termination or production of a truncated protein & rapid instability of SORD gene mRNA [RNA decay].

Conclusion: SORD-CMT2 has been reported in Egyptian families with HPN. Phenotypic spectrum included sensorimotor peripheral neuropathy, SNHL, anosmia, autonomic neuropathy and diabetes. SORD deficiency is expected to result in increased blood levels of sorbitol causing axonal damage to peripheral nerves. This provides information to screen Egyptian population with CMT2 for SORD gene mutations, opportunity for genetic counseling & participation of patients in clinical trials of investigational new treatments which target the underlying cause of SORD deficiency.

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GENETICS OF COMPLEX DISEASES

GENOMIC CHARACTERISATION OF KNEE OSTEOARTHRITIS

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Introduction: Knee osteoarthritis (kOA) is a major cause of disability in the elderly with a global prevalence of 16% and 22-39% in India. Major risk factors for kOA are aging, diet, lifestyle, and most importantly genetic modality. To date, genetic predisposition is not fully understood and also there is a lack of genetic studies from India.

Materials and methods: In this study, we have sequenced kOA patients through exome sequencing to decipher the associated genetic variation. 70 kOA patients were screened clinically and confirmed the diagnosis through radio-graphical intervention. Clinical history, demography & 5mL blood sample were collected. DNA was isolated and subjected to exome sequencing (100X) in the Illumina Novaseq 6000 platform after preparing libraries using nextera exome enrichment kit. Through mRNA expression studies, these candidate's gene levels were compared with healthy controls to correlate with genetic profile.

Results: Germline variants were identified in previously known kOA candidate genes such as *BTNL2 and HLA-DPB1*, and several other genes with potentially deleterious variants for OA. In an independent filtering for casual variants that were associated with OA or related bone disorders based on clinvar database, we found previously reported variants in genes *NACA2, TLR10, GLE1 & MATN3* having high minor allele frequency as compared to 1000genomes and SAS data frequency. Interestingly, whole-exome analysis demonstrated that most samples showed multiple modifier variants whereas novel or rare variants were specific to the individuals. The mRNA expression for these candidate genes were either upregulated or downregulated significantly and showed distinct gene expression profiles.

Conclusion: Our findings indicate that OA patients harbor potentially deleterious causative germline variants in genes that function in several candidate causative pathways including immune responses, inflammatory and cartilage degradation. Genetic testing for a wider variety of OA-predisposition genes could provide a better screening approach for high-risk groups of osteoarthritis.

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GENETICS OF COMPLEX DISEASES

Enhancing Type 2 Diabetes Risk Stratification: A Comprehensive Ensemble Learning Approach Integrating Multi-Trait Polygenic Scores

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Background: Type 2 diabetes (T2D) is a prevalent condition, with over 500 genetic variants identified through genome-wide association studies. Despite these findings, translating genetic associations into effective predictive models for disease risk prediction remains challenging. Polygenic scores (PRS), which aggregate the effects of multiple genetic variants, offer enhanced capabilities in detecting disease susceptibility and stratifying high-risk individuals. PRS can serve as valuable outcome predictors in clinical applications and screening programs by assessing an individual`s overall genetic risk for a specific trait. Integrating information from genetically correlated traits into PRS models has shown potential to improve prediction accuracy and power.

Objective: This study aimed to identify genomic variants associated with T2D risk in the Qatar Biobank (QBB) cohort and develop machine learning (ML) models incorporating multiple PRS predictors for enhanced risk evaluation.

Methods: Genome-wide association studies (GWASes) were conducted for 12 T2D-related traits to identify genomic variants linked to diabetes clinical characteristics. The study involved 14,278 QBB participants, forming the basis for GWAS and PRS development. Multiple weighted PRS models were constructed using associated variants for each trait, and ML models evaluated the combined risk from all 12 predictors.

Results: The predictive models demonstrated robust ability in stratifying T2D cases from controls, with the best-performing model achieving an accuracy of 0.76, an area under the receiver operating characteristic curve (AUC) of 0.84, an area under the precision-recall curve (AUC-PR) of 0.88, and an F1 score of 0.766.

Conclusion: PRS models exhibit the potential to identify individuals at risk of developing T2D and associated complications within our population. Stratifying individuals with T2D into distinct risk groups can aid in personalized preventive interventions and targeted healthcare strategies.

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GENETICS OF INFECTIOUS DISEASES

A GENETIC AND IMMUNOLOGICAL PERSPECTIVE OF THE COVID-19 PANDEMIC IN SOUTH AFRICA

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Very few viruses have led to a global pandemic, within the modern era, to the extent observed, as COVID-19. Despite the widespread death observed across the globe, there was vast heterogeneity in susceptibility and severity of the disease. Reports have shown an intricate interplay between the environment, human genetics and immunogenicity on severity of COVID-19 disease. In this study, we investigate the contribution of specific genes, immune responses, and other potential contributing factors (including comorbidities, vaccines, etc.) on the influence and impact on the susceptibility and disease severity. We therefore examined, (1) the effect of comorbidities and other infectious disease on the outcome of disease; (2) the frequency of specific polymorphisms within the SARS-CoV-2 receptors genes and their effect on gene-expression and SARS-CoV-2 viral loads; (3) and the longevity of SARS-CoV-2 antibodies and the impact of vaccinations drives on the severity of disease. Participants recruited into the study consisted of Black, Indian, and White South African participants who were PCR-confirmed positives for SARS-CoV-2. Participants were followed up longitudinally across three time-points post-infection. Co-infection, vaccination, medication taken, and other clinical characteristics were recorded at each of the visits. The presence of four polymorphisms within the SARS-CoV-2 receptor and co-receptor genes (rs2285666 [ACE2], rs12329760 [TMPRSS2], rs10080 [NRP1] and rs8259 [CD147]) were determined for each participant. We next measured SARS-CoV-2 viral-load using droplet digital PCR during time of infection nasopharyngeal swabs. Lastly, SARS-CoV-2 specific IgM and IgG antibody levels were determined using ELISA. We demonstrated that a combination of factors contribute to the diverse COVID-19 disease severity observed across ethnic groups. Our study shows (1) vaccination, Ivermectin usage and co-infections influence COVID-19 severity and susceptibility. (2) ACE2 and NRP1 polymorphisms (rs2285666) and (rs10080) respectively significantly associated with increased SARS-CoV-2 viral load and more severe COVID-19 outcome in specific ethnic groups. (3) SARS-CoV-2 specific antibodies, IgM and IgG, are observed to demonstrate ethnic-specific differences in antibody levels. Our study provides a unique insight into the genetic, immunological and environmental factors that influence COVID-19 diseases severity across ethnicities in South Africa. This will assist with designing superior therapeutic interventions against future pandemics.

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RARE DISEASES

A COMMUNITY BASED STUDY ON PREVALENCE OF INTELLECTUAL DISABILITY(1-18YEARS) IN TIRUPATTUR DISTRICT, TAMILNADU, INDIA.

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Pugazh Centre for Genetic Counseling is situated in Tirupattur district in the State of Tamil Nadu, South India. It is the first community based Genetic Counseling centre with the Government approval.

Objectives of the Study: To find the prevalence of intellectually disabled children (1-18years) in Tirupattur District.

Methodology:

An extensive study on rare diseases was conducted from 2021 to 2023 using a specially designed case sheet. All the children are physically identified from schools, Early Intervention centre, Day care centres and houses through door to door step from all the 6 health blocks comprising of 208 village panchayats, 5 municipalities and 3 town panchayats. After inclusion of kids in our study we reviewed previous medical records, took detailed pedigree analysis, provided Genetic counseling to their families for the prevention of further damage in the children and pre-pregnancy genetic counseling for a healthy baby.

Results and Discussion:

Population size – Rural 217133, Urban 92422 and Tribal 13332 (Total 322887 children).

Total number of disabled children 1481. Out of which intellectually disabled are 743, Autism spectrum disorder 21, hearing impaired 158, visually impaired 59, and intellectual disability with multisystem involvement 105.

Family history of Intellectual disability in 16%

Consanguinity in 63%. Out of which Second degree consanguinity in 24% and third degree consanguinity in 39%

Antenatal history medication taken for acute or chronic conditions in first trimester is 3%

Antenatal genetic testing and /or counseling: practically nil.

Conclusion:

The state has NO database of Genetic, endocrine or metabolic disorders that occur at birth, for the 11 lakh children born in the state every year. Such conditions lead to death within the first four weeks, or it stunts growth and causes irreparable damages to the brain. Genetics conditions are not RARE it seems to be under diagnosed or ignored. The present study will create awareness for maintaining community genetics workup to prevent intellectual disability.

Preventable measures are,

Community based awareness program about Genetic disorders,
New Born Screening (NBS) for all babies,
Improving Maternal and Child Health.

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RARE DISEASES

Von-Hipple Lindau Syndrome - A case report from INDIA

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Von-Hipple Lindau (VHL) is an autosomally dominant inherited condition characterized by tumors of various organs such as retinal, CNS hemangioblastoma, renal carcinoma or pancreatic tumors etc. The prevalence of this disorder is 1 in 36000 live births. Tumors may be benign or malignant including various organs. VHL, usually show its symptoms by 65 years of age. The disease is due to VHL gene present on chromosome 3 which is mainly a tumor suppressing gene. The VHL gene consists of three exons and code for VHL protein which is a glycan anchored protein helps in signal transduction.

A 23 year old female presented to our department with complaints of visual disturbances and abdominal pain for which she underwent ophthalmological evaluation and was diagnosed to have retinal angiomas and ultrasonography abdomen revealed multiple pancreatic cysts largest 3.7*4.2 cm9s and cystic lesion in left kidney. In view of bilateral retinal angiomas and pancreatic cyst clinical suspicion of VHL syndrome was kept and magnetic resonance of brain was done which show right cerebellar hemangioblastoma, renal function test were normal with blood urea of 20 and serum creatinine of 0.7 mg/dl, with uric acid level of 5.1 mg/dl. For cerebellar hemangioblastoma she was operated with mini-supraorbital craniotomy and excision, medium pressure ventriculoperitoneal shunt placement was done. There was positive history of renal cell carcinoma in her father. With the above constellation of findings and presence of a positive family history clinical diagnosis of VHL syndrome was kept and targeted NGS was done which showed a heterozygous missense variation in exon 1 of VHL gene on chromosome 3: VHL:c.333CG:p.(Ser111Arg), this variant has been previously reported as pathogenic for VHL syndrome. The lady is in our follow up and screening in children for the same variant was planned.

Periodic screening of eyes, hearing evaluation, abdominal screening, neuroimaging and screening for other cancers like pheochromocytoma has been been recommended in all cases of VHL.

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MICROBIOME AND METAGENOMICS

The Women4Health cohort: a multi-omics approach to understand the role of microbiome in women's metabolism.

PhD Fabio Busonero[1], Stefania Lenarduzzi[2], Francesca Crobu[1], Roberta Maria Gentile[3], Andrea Carta[4], Francesco Cracco[3], Andrea Maschio[1], Silvia Camarda[3], Michele Marongiu[1], Daniela Zanetti[1], Claudio Conversano[1,4], Giovanni Di Lorenzo[2], Daniela Mazzà[2], Francesco De Seta[2], Giorgia Girotto[2,3], Serena Sanna[1,5]

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Introduction: Cardiovascular and metabolic disorders (CMD) are characterized by dysfunction of glycemic and/or lipid metabolism and result from both genetic and environmental factors. Relevant differences in clinical manifestations, severity and progression exist between sexes, the underlying mechanisms of which are largely unknown. Large genetic studies have shown that sex dimorphism can be only minimally explained by genetic. Among the environmental factors, attention has been given recently to the gut microbiome in the onset of CMD and in the regulation of risk factors, but its role in sex dimorphism has not yet been explored.

Objectives: We aimed to investigate the role of female sex hormones in influencing the gut microbiome and thus its effect on host lipid and glycemic metabolism, mechanism that could explain the major differences observed in CMD around menopause age.

Methods: To do so, we have set up the Women4Health cohort, an observational short-term longitudinal study of 300 women aged 18 to 45 years old. At weekly appointments during a natural menstrual cycle, we directly measure sex hormones, blood biochemistry parameters (lipids, fasting glucose and insulin, transaminases) and record relevant metadata (such as daily diet, medication use, bowel habits). Furthermore, we collect several biological specimens to characterize the host's genome, the gut microbiome, the fecal metabolome, and the plasma proteome. We also collect biological specimens to characterize the vaginal microbiome, the unique microbiome ecosystem of females, to evaluate its direct or indirect role in regulating host metabolism.

Results: Preliminary data in the firstly 50 enrolled volunteers already indicate that clinical parameters, such as insulin, lipids and transaminases significantly change during four phases of the menstrual cycle.

Conclusions: Preliminary findings support the feasibility of this study design to explore the impact of female's sex hormones variations on microbiome's composition and women's metabolism. We will describe the multi-omics strategy and design of the Women4Health cohort.

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RARE DISEASES

Unique genetic architecture of ALS in the isolated island population of Malta

Professor Sylvana Tabone[1,2,3], Maia Farrugia Wismayer[1,2,3], Paul Herrera[1,2,3], **Ruben J Cauchi**[1,2,3]

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Genetic risk for amyotrophic lateral sclerosis (ALS) is highly elevated in genetic isolates, like the island population of Malta in the south of Europe, providing a unique opportunity to investigate the aetiology of this rare disease. Founded in 5000 BC by settlers crossing the sea to Malta from neighbouring Sicily, the genomic insularity of Maltese from the rest of mainland Europe has been apparent since Neolithic times based on recent analysis of ancient and modern genomes. Here we characterize the genetic profile of the largest series of Maltese ALS patients identified to date by Malta's National ALS Registry and Biobank, and correlate this data with the clinical phenotype. Whole genomes of cases were first mined for rare variants in known ALS causative or risk genes. Potentially damaging variants or repeat expansions were identified in more than 30% of all patients with this underscoring one of the most populations with a high percentage of genetically explained cases in Europe. In support, the rate of familial ALS in Malta is higher than the European median and the percentage of juvenile ALS (jALS) cases was higher than that expected for the population size. Notably, damaging variants in global major ALS genes C9orf72, SOD1, TARDBP and FUS were either absent or rare in ALS patients of Maltese ancestry. The most frequently affected genes were ALS2, DAO, SETX and SPG11, which are infrequently linked to ALS in Europeans. Newly identified compound heterozygous mutations in the ALS2 gene were linked to jALS. Importantly, through a non-gene candidate mining pipeline involving Maltese elderly controls as the reference genome we identified ultrarare damaging variants in a gene that we link for the first time to ALS and whose predicted function in vesicle transport function ties well with pathways disrupted in ALS. Compound heterozygous mutations in this gene are a cause of the disease in the highest percentage of Maltese ALS patients investigated to date. Present work is focused on investigating dysfunction of this novel ALS gene in animal models. Our study underscores the use of genetic isolates to map novel genes contributing to the missing heritability of ALS.

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RARE DISEASES

A STRUCTURAL VARIANT CAUSES PETTIGREW SYNDROME - NEW FINDINGS BY USE OF LONG-READ GENOMIC SEQUENCING

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Background

Pettigrew syndrome, first described in 1991, is a very rare (about 50 affected patients) intellectual disability syndrome with a wide variety of additional features such a choreoathetosis, hydrocephalus, Dandy-Walker malformation, seizures and iron or calcium deposits in the brain. It shows high variability in expression both between and within families. This syndrome is caused by changes in the X-chromosomal AP1S2 gene, known since 2014. This gene encodes for the sigma-2 subunit of the heterotetrameric adaptor protein 1 (AP1) complex found in the cytosolic side of coated vesicle in the Golgi compartment. AP1 mediates the recruitment of clathrin and the recognition of sorting signals of transmembrane receptors. Known pathogenic variants described in ClinVar are nonsense, frameshift or intronic (considering splice sites) variants.

Long-read genomic sequencing by PacBio`s Revio utilizes single-molecule, real-time (SMRT) technology. This platform produces extended DNA reads, enabling the sequencing of complex genomic regions, structural variations, and full-length transcripts.

Case

Our case is a 7-year-old boy with severe intellectual disability, no speech, facial dysmorphism, psychomotor developmental delay, a funnel chest and hypotonia. The parents are both healthy.

Trio-exome analysis was indicative in some reads for a hemizygous deletion/insertion of an unknown sequence in the first coding exon of AP1S2 gene without any reference to size and origin of this aberration.

To elucidate in more detail this variation long-read genomic sequencing was performed. It revealed that the aberration is a 138kb inversion with the breakpoints proximally in the CA5BP1 gene and distally in exon 1 of the AP1S2 gene.

Conclusion

The inversion involving AP1S2 was inherited from the mother, who is healthy and of normal intelligence. Previous studies have shown that females heterozygous for AP1S2 variants are usually asymptomatic and show normal X-inactivation patterns in blood DNA.

In the described patient, it was possible to detect the origin of his clinical phenotype, mainly due to the new technology of long-read genomic sequencing, which allows detailed analysis of complex genomic regions, aiding precise variant characterization for better understanding of genetic disorders. Here we could elucidate the complex aberration, which represented an inversion of part of the AP1S2 gene.

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PANGENOMES AND GENOMIC DIVERSITY

RECOMBINATION OF REPEAT ELEMENTS GENERATES SOMATIC COMPLEXITY IN HUMAN GENOMES

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Non-allelic recombination between homologous repetitive elements contributes to evolution and human genetic disorders. In our previous work we have revealed that the somatic recombination of Alu and L1 elements is widespread in the human genome. We uncovered tissue-specific recombination hallmarks, recombination hotspots and cell-type specific dynamics of recombination during differentiation. We furthermore identified tissue and disease-specific alterations of recombination profiles in Parkinson`s and Alzheimer`s disease, suggesting a link between retroelement recombination and genomic instability in neurodegeneration. In our new data we show that ablating the non-homologous ends joining DNA repair pathway (NHEJ) surprisingly affects genome-wide recombination profiles in cultured cells. Moreover, our new analyses of ultra-deep, PCR-free and capture-free WGS datasets we improve on our estimates of recombination in single cell genomes to show that recombination of repeats is even more frequent than previously anticipated, and it constitutes the most abundant source of somatic structural variants in the human genome.

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RARE DISEASES

A Case Report of 10q24.32 Microduplication associated with Split Hand/Foot Malformation (SHFM) in Prenatal Diagnosis

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Introduction: Split Hand/Foot Malformation (SHFM), commonly known as ectrodactyly, denotes a rare genetic disorder characterized by limb malformations. Currently, six loci associated with non-syndromic SHFM are recognized: SHFM1 at 7q21.2q22.1 (DLX5 gene), SHFM2 at Xq26, SHFM3 at 10q24q25, SHFM4 at 3q27 (TP63 gene), SHFM5 at 2q31, and SHFM6 as a result of variants in WNT10B (chromosome 12q13). Its prevalence occurs in 1 in 8.500–25.000 newborns and accounts for around 15% of all limb reduction defects. In this study, a 41-year-old pregnant woman with hand and foot anomalies underwent fetal ultrasound screening. The foetus showed higher nuchal translucency of 2.5 mm and a typical SHFM.

Method. Cytogenetic karyotype on foetus was performed as first-tier test, followed by molecular karyotyping using Comparative Genomic Hybridization Array (aCGH).

Results: aCGH performed on the fetus showed a 10q24.32 microduplication (chr10:101227671-101630125), comprising the entire extra copies of BTRC, DPCD, and POLL genes and a partial extra copy of the FBXW4 and LBX1 genes, known to be involved in limb development. Subsequent aCGH analysis of both parents was performed, confirming maternal transmission of the same 10q24.32 microduplication. ClinGen Database was used to define the genes' pathogenicity score revealing FBXW4 gene as limited associated with SHFM type 3 disease.

Conclusion: SHFM3 is characterized by a wide clinical spectrum of severity, even within the same family, ranging from a mild hand defect to an extreme limb aberration, some of which incompatible with life. In the present study, in both foetus and the mother, the presence of a 402 Kb duplication in chromosome 10 was identified, supporting the link between 10q24.32 microduplication and SHFM3. It seems plausible that the complexity of SHFM3 involves a broader interplay of genes, urging a deeper exploration to elucidate the intricate genetic mechanisms underlying the variable expressivity of SHFM3.

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ETHICAL, LEGAL AND SOCIAL ISSUES ON HUMAN GENETICS

Who chooses to undergo DNA testing for hereditary tumor risk syndromes – experience from a Bulgarian center

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Background: Hereditary tumor risk syndromes counseling is already a part of the routine care in most genetic centers. This study aims to evaluate the group of patients opting in for DNA testing for probable tumor risk syndromes in our country, minding the financial burden it poses and the initial indications and restrictions of these tests. Thus, we aim to identify demographic and socio-economic factors associated with the proneness of patients to such analyses.

Methods: We divided the patients counseled for hereditary tumor risk syndromes in 4 years (2020-2023) into two groups – with and without further DNA tests. The two groups were assessed based on age, sex, residence, and financing of prior genetic counseling (GC) - self-paid or free. We also evaluated the share of patients undergoing WES for the same period with various indications that prefer knowing about secondary findings, including hereditary cancer syndromes.

Results: From all 123 patients, 79 (64.2%) were self-paid, and 44 (35.8%) were offered a free GC. 62 (50.4%) proceeded with DNA testing with a progressively increasing number during the studied period. DNA testing following GC was statistically significantly more common for the patients with the self-paid GC - 56 (70.8%), compared to the free GC cases - 6 (13.6%). The group that underwent DNA tests was predominantly women (65.9%) with a younger mean age, 47y vs 53y for non-testers. 52 (83.9%) patients with further testing were from the city of our genetic center – Varna, 3rd in size and one of few in Bulgaria with a genetic center. Also, 91.6% preferred the most expanded DNA tests. Additionally, all 51 patients undergoing WES opted in for secondary findings.

Conclusions: Our study shows a general tendency for an increase in patients` willingness to undergo testing for hereditary tumor risk syndromes. Unexpectedly, prior GC self-financing is not a limiting but a predisposing factor. Being young, female, and living in a big city with a nearby genetic center are characteristics that are associated with proactivity regarding such prophylaxis. Still, additional studies are needed to confirm a causal relationship instead of an observed association.

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NON-CODING RNA GENES

Exploring cardiac exosomal RNAs of acute myocardial infarction

Exploring cardiac exosomal RNAs of acute myocardia Jung-Won Choi[1], Seung Eun Jung[1], Sang Woo Kim[2]

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Myocardial infarction (MI), often the first symptom of coronary artery disease (CAD), is a leading cause of death and disability. Acute myocardial infarction (AMI), a major cardiovas-cular disease, significantly contributes to global morbidity and mortality. Understanding AMI`s complex pathophysiology is crucial for developing innovative therapeutic strategies. Exosomal RNAs (exoRNA) within cardiac tissues, particularly microRNAs (miRNAs), play a critical role in intercellular communication and pathophysiological processes in AMI. This study aims to delineate the exoRNAs landscape, especially miRNAs, in animal models using high-throughput sequencing. Our approach included sequencing analysis to identify significant miRNAs in AMI, followed by validation of the functions of selected miRNAs through in vitro studies involving primary cardiomyocytes and fibroblasts. We identified numerous differentially expressed miRNAs in AMI and validated the functions of 20 selected miRNAs through in vitro studies with primary cardiomyocytes and fibroblasts. Our research enhances understanding of post-AMI molecular changes in cardiac tissues and investigates the potential of exoRNAs as bi-omarkers or therapeutic targets. These findings offer new insights into AMI`s molecular mecha-nisms, paving the way for RNA-based diagnostics and therapeutics and therapies and contributing to the advancement of cardiovascular medicine.

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NON-CODING RNA GENES

HUMAN LNCRNAS HARBOR SHARED MODULES

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Eukaryotic proteins are often composed of domains that are modularly associated in different numbers and types. It is well known that a strong association is present between exons and domain sequences, this association being also the basis for the exonic theory for the origin of eukaryotic genes. We analyzed human long non-coding RNA genes seeking for a similar modular organization of the non coding transcriptome by comparing all known non-repetitive human lncRNA exons and identifying 340 pairs of them sharing high sequence and/or secondary structure similarity but embedded in a dissimilar sequence context. We grouped these pairs in 106 clusters based on their reciprocal similarities. These shared lncRNA modules are highly conserved between humans and the four great ape species, display evidence of purifying selection and likely arose as a result of recent segmental duplications. Overall our results indicate that lncRNA genes may have a similar modular organization as protein coding genes, and suggest a potential mechanism for the evolution of this architecture. These observations could also impact the way we define and annotate human non-coding genes.

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CANCER GENOMICS

A Population Genomics survey for Hereditary Breast Cancer

A Population Genomics survey for Hereditary Breast Antonio Grimaldi[1,2], Rosa De Santis[1], Tonya Fusco[1], Davide Cacchiarelli[1,3]
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Hereditary Breast and Ovarian Cancer Syndrome (HBOC) is a genetic condition characterized by a higher predisposition to breast or ovarian cancer. It affects males and females, accounting for about 5 to 10% of all breast cancer and up to 20% of all ovarian cancer cases worldwide. HBOC syndrome is usually associated with early tumour onset, usually before 50, and, while typically associated with breast and ovarian cancers, it can also be causative of other malignancies, including melanoma, prostate, and pancreatic cancer. The main genetic determinants of HBOC syndrome are mutations in BRCA1 and BRCA2. Mutations in BRCA1 lead to a 45-87% and 39-63% risk of developing breast and ovarian cancer by age 70, respectively. Similarly, BRCA2-associated risks are 38-84% and 16-27%.
Being able to rapidly and cost-effectively screen patients for the presence of mutations in BRCA1/2 would be thus a powerful tool for disease prevention and early treatment. Unfortunately, current approaches heavily rely on expensive technologies or low throughput. Here we propose an amplicon-based strategy relying on only two PCR steps and able to specifically explore the genomic sequences of BRCA1/2 and other important determinants of HBOC, including CHECK2 and PALB2. In addition to this and to further decrease sequencing costs while increasing the throughput, we set up an experimental protocol based on minimizing reaction volumes and minimal operator hands-on time. In conclusion, we seek to generate a new approach for the dissection and the screening of pathogenic genetic variants that can easily be applied to a large cohort of patients.

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CANCER GENOMICS

Characterizing Paclitaxel-Responsive APC mutations in H460 Lung cancer cell variants through Targeted Next-Generation Sequencing (T-NGS)

Identifying Paclitaxel-Responsive APC Mutations in Hye Won Choi[1], Kyeong Man Hong[1], Young Ho Kim[1], Eun-Kyung Kang[1]
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Background: There have been numerous studies on the heterogeneity of cancer cells in a patient or in a cancer cell line. The variants hovering specific drug-responsive mutations, in which the rest of changes are the same as parent cells, would provide a valuable tool to screen drug-responsive markers, and to study the mechanism of drug responsiveness.

Methods: As a proof of concept, the changes of mutations in H460 non-small cell lung cancer cell were monitored by targeted next-generation sequencing (T-NGS) method after treatment of paclitaxel. Variants with paclitaxel-responsive mutations cloned from the parent H460 cells, and the changes in the ratio of mutation to null were monitored in 3D cultures and an animal experiment by a single-base extension method.

Results: We found several mutations which are responsive to paclitaxel treatment from the screening by T-NGS, and a mutation in APC were selected as a candidate marker for paclitaxel responsiveness. The mutant to null peak ratio was significantly lower in mixed 3D cultures and animal experiments, suggesting that the mutation in APC should be a drug-sensitive marker.

Conclusion: Monitoring the changes of the heterogenous mutations in cancer cells would be a useful tool to screen drug-responsive mutations, and a mutation in APC is a paclitaxel-sensitive marker.

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RARE DISEASES

THE ROLE OF ARRAY-CGH ANALYSIS IN THE DIAGNOSIS OF RARE DISEASES: A SINGLE-CENTER EXPERIENCE

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Introduction

The prevalence of rare diseases worldwide is estimated to be between 3.5% and 6%, with over 8,000 identified conditions. In rare diseases, difficulties in diagnosis can arise due to the inability to identify the responsible gene or chromosomal region, or other genetic etiology. Array-CGH analysis is an important tool in the diagnosis of rare diseases due to its ability to identify defined microdeletion-duplication syndromes, unique copy number variations in individuals, major chromosomal abnormalities, chromosomal numerical anomalies, single gene disorders, imprinting disorders; providing insight into mosaicism, and detecting loss of heterozygosity. We aim to present our center`s experience with array-CGH in rare diseases.

Methods and Material

Molecular karyotyping was conducted on the Illumina iScan System 300K (in 72 patients) and Illumina iScan System 700K (in 623 patients) microarray platforms for 695 patients who presented with various indications at the Çanakkale Onsekiz Mart University Genetic Diagnosis and Treatment Center between 2020 and 2023.

Results

Rare diseases were diagnosed in 47 out of the 695 patients we analyzed (%6.7). Copy number variations were detected in 10 patients, with a single gene (SHANK3, CHRNA7, GLI3, HNF1B, NRXN1, ABCG2, NF1, HBA1, BHLHA9) responsible for the clinical manifestations. Copy number variations were detected in rare regions among 14 patients, with sizes ranging from 145 KB to 7.7 MB, along with identified variations in microdeletion/duplication syndrome regions including 22q11.2 microdeletion syndrome in 4 patients, Prader-Willi/Angelman syndrome in 3 patients, 3q29 microduplication syndrome in 4 patients, and other syndromes found in 1 patient each such as Phelan McDermid syndrome, 1q21.1 duplication syndrome, 9q31.1q31.3 microdeletion syndrome, Cat-eye syndrome, 6q25 microdeletion syndrome, and 8q21.11 microdeletion syndrome. Additionally, major numerical and structural chromosomal anomalies, such as trisomy 9 p, partial monosomy 11q, 6q25 microdeletion syndrome, Turner syndrome, and derivative Y chromosome, were detected in 7 of these patients, which can also be identified through chromosome analysis.

Conclusion

In today`s context where sequencing technologies have emerged as prominent tools for diagnosing rare diseases, the significance of array-CGH technology remains crucial in diagnosing rare diseases due to its ability to diagnose genetic disorders arising from various mechanisms.

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RARE DISEASES

Epigenomic Biomarkers as Targets for Therapeutic Interventions in Sickle Cell Disease (SCD) pain and overt stroke complications.

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SCD is a rare hereditary blood disorder caused by a mutation in the β-globin gene, leading to the production of abnormal hemoglobin. This results in the deformation of red blood cells into a sickle shape, leading to various complications, including chronic pain, multi-organ damage, stroke, poor quality of life and reduced life expectancy. While advances have been made in understanding the genetic basis of SCD, emerging evidence suggests that epigenetic modifications, such as DNA methylation, play a critical role in the pathogenesis of the disease and has potential for identifying prognostic biomarkers and therapeutic targets. Epigenetics refers to modifications in gene expression patterns that are not caused by changes in the underlying DNA sequence. Epigenomic biomarkers, such as DNA methylation patterns and histone modifications, have been shown to play a crucial role in regulating gene expression The proposed research aims to explore the potential use of epigenomic biomarkers as targets for therapeutic interventions in SCD pain and overt stroke complications.

This research will involve a multi-disciplinary approach, combining genomics, epigenomics, and bioinformatic data analysis. The proposed research will involve collecting blood samples from SCD patients with pain episodes and overt stroke complications, as well as healthy individuals as control. These samples will be subjected to comprehensive epigenomic profiling, including DNA methylation analysis and histone modification analysis. The data obtained from this profiling will be analyzed using advanced bioinformatics tools to identify differentially methylated regions and modified histone marks that are associated with SCD pain and overt stroke complications. This research will provide valuable insights into the epigenetic basis of SCD pain and overt stroke complications, potentially leading to the identification of novel therapeutic targets for these debilitating symptoms. Furthermore, understanding the epigenetic mechanisms underlying SCD pain and stroke complications can pave the way for personalized medicine approaches in managing this condition facilitating early intervention, targeted treatment strategies, and improved health outcomes for individuals with sickle cell disease.

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CANCER GENOMICS

A NEW APPROACH FOR BLADDER CANCER (BC) DIAGNOSIS BASED ON TARGETED SEQUENCING

A new approach for Bladder Cancer (BC) diagnosis Tonya Fusco[1,2], Antonio Grimaldi[1,2], Rosa De Santis[1], Davide Cacchiarelli[1,2,3]
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Bladder cancer (BC) is the most common neoplasm of the urinary system. It represents about 70% of the forms of cancer affecting the urinary tract and about 3% of all cancers. This tumor is three times more frequent in men than in females, with an average onset between 60 and 70 years of age. As other neoplasms, BC is not directly linked to a specific germline mutation. Instead, its progression and formation is driven by a complex set of somatic variants that affect a heterogeneous group of genes, that are involved in the regulation of cell cycle, like TP53, and in cell proliferation, like FGFR3. The main treatment of this tumor is the surgical removal, which, however, does not guarantee persistent healing because BC has a very high recurrence rate, between 50% and 80%. For this reason it is very important to have a method to monitor patients even in the years following the removal of the tumor. Today the cystoscopy is considered the gold standard for the diagnosis and for follow up tests. However, this technique is very invasive and it could be interesting to apply an alternative and less invasive strategy. From this point of view, the Next generation Sequencing technologies could be an excellent solution. Here, we propose a targeted sequencing, by focusing on a small set of genes, like a powerful tool for BC prevention and early treatment, but also for the follow up, using urine as starting sample. We decide to apply an amplicon-based strategy relying on only two PCR steps and able to specifically explore the genomic sequences of TP53, FGFR3 and other important determinants of BC, including RB1, ERBB2 and PIK3CA. With this strategy, we are able to: process many samples in parallel and obtain a high number of reads for the analyzed regions, minimizing reaction volumes, costs and operator hands-on time. In conclusion, based on our preliminary achievement, we hope to generate a new approach for BC diagnosis and follow up controls, based on targeted sequencing that is more tolerable and it can easily be applied to a large cohort of patients.

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GENETICS OF INFECTIOUS DISEASES

UNIQUE GENOME-WIDE METHYLATION PATTERNS WITHIN HIV DISEASE

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Despite more than 40 years of research, HIV is still a major health concern. Advances in the field have helped us understand various aspects of the virus and this has led to the development of antiretroviral therapy (ART), which has extended life expectancy and lowered the number of new infections. However, getting rid of HIV with ART in a large endemic is a costly and complex activity. Furthermore, drug resistance is rising at alarming rates and new treatment options are promising but have serious side effects. Thus, it is increasingly important to focus attention on developing an HIV vaccine and cure. HIV host genetics has made significant advances in HIV cure research. In our recent work, we demonstrated the importance of the epigenetic mechanism, DNA methylation, plays on HIV disease. In this study, we explored a rare cohort of females (n=120) followed up over 15 years from pre-HIV infection to post-HIV infection and throughout various fiebig stages. The treatment naïve individuals were then followed up post-ART initiation. Using this unique cohort, we explored the role genome-wide methylation has on HIV infection and disease progression, using the Infinium MethylationEpicv2.0 array, which covers 850,000 methylation sites. We report unique DNA methylation patterns from the HIV-negative timepoint compared to the HIV-positive timepoint in the same patients. We identified 8 DMPs across pre-infection and post-infection and 14,274 DMPs between pre-treatment and post-treatment timepoints. Clustering of CpG probes using Independent Component Analysis was used to assess functional enrichment. Viral loads and CD4+ T cell counts were taken into consideration in the construction of ICA clusters. Both viral load and CD4 count were shown to be associated with one ICA module. Gene Ontology enrichment was performed on this module using the genes linked to the CpG probes, which revealed associations with Interleukin-1 regulation, viral entry/viral processes, histone modifications and B-cell proliferation. Data from this study shows that genome methylation patterns vary across different stages of HIV infection and that these methylation patterns might have an impact on controlling HIV infection and may prove crucial for the development of future therapeutic interventions aimed at HIV-1 cure.

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PRECISION HEALTH

ADVANCING INSIGHTS INTO HEARING LOSS: UNVEILING NOVEL VARIANTS AND PATHOGENIC PATHWAYS THROUGH A STRATEGIC MULTISTEP GENETIC ANALYSIS AND PRE-CLINICAL RESEARCH IN ZEBRAFISH

PhD Paula Inés Buonfiglio[1], Carlos David Bruque[2], Mariela Pace[1], Lucia Salatino[3], Vanesa Lotersztein[4], Sebastián Menazzi[5], Paola Plazas[3], Ana Belén Elgoyhen[1,3], Viviana Dalamón[1]

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Hearing loss (HL) affects nearly 10% of the global population, with over half of the cases due to genetic factors. Congenital HL is observed in 1 in 500-1000 newborns, often manifesting as non-syndromic cases (70%) and with an autosomal recessive mode of inheritance (80%). It is mostly related to genetic variants in GJB2 and GJB6 genes, however, over 100 genes are associated with HL. Whole-exome sequencing (WES) technique has emerged as a cost-effective molecular diagnostic tool to analyze all the related genes at once. However, challenges persist, particularly in detecting novel variants as missense changes, which may hinder the genotype-to-phenotype correlations.

This study aimed to uncover the genetic causes of HL in a cohort from Argentina. Additionally, in silico and in vivo analyses were conducted to explore the impact of novel variants on hair cell function using the zebrafish model.

Sanger Sequencing and GAP-PCR in GJB2 and GJB6 diagnosed 15.5% of sporadic cases and 36% of familial cases among 650 patients. Further screening of 50 undiagnosed patients with moderate HL for STRC gene deletions using Multiplex ligation-dependent probe amplification led to a 6% diagnosis rate. Subsequently, WES analysis was performed in 50 families, resulting in a 44% diagnosis rate, with half of the identified variants being novel. A missense variant in MYO6 gene, detected in a family with postlingual HL, underwent protein modeling with AlphaFold2, confirming its pathogenic nature. Functional validation in zebrafish demonstrated the variant`s deleterious effect on stereocilia mobility in neuromasts, impacting the auditory system function.

The importance of zebrafish in precision medicine was highlighted, as the model allowed for a nuanced understanding of the functional implications of identified genetic variants. Integrating zebrafish analyses into diagnostic frameworks enhances the precision and clinical relevance of genetic findings, paving the way for more targeted therapeutic interventions in the field of HL. This work demonstrates the suitability of our algorithm for HL genetic diagnosis in our cohort. The results underscore the importance of a combined strategy, integrating in silico and in vivo studies to identify candidate variants, analyze their pathogenicity, and enhance understanding of the physiopathology of hearing impairment.

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GENETICS OF COMPLEX DISEASES

CAUSAL ASSOCIATIONS BETWEEN LIVER BIOMARKERS AND TYPE 2 DIABETES MELLITUS IN AFRICAN ANCESTRY INDIVIDUALS: A BIDIRECTIONAL MENDELIAN RANDOMIZATION STUDY

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Observational studies have shown that serum concentrations of circulating liver enzymes are linked to Type 2 Diabetes Mellitus (T2DM). However, the direction of this causality whether liver biomarkers cause, result, or the association is confounded remains unknown. In this study, we undertook a bidirectional Mendelian Randomization study to delineate the causal pathway between Liver dysfunction proxied by liver biomarkers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin and bilirubin with T2DM in African Ancestry Individuals. Using liver biomarkers from the African Partnership for Chronic Diseases Research (APCDR) (N =14,000) and T2DM from African American participants in the Million Veteran Programme (MVP) (Ncases = 23,305 and Ncontrols = 30,140), we found that genetically predicted liver biomarkers were not significantly associated with T2DM risk. ALT= Odd ratio (OR), 1.06, 95% Confidence interval (CI), 0.56-2.03; P = 0.83), albumin = OR, 0.82, CI, 0.58-1.14; P = 0.25), ALP = OR, 0.93, CI, 0.79-1.09; P = 0.40), AST= OR, 1.09, CI, 0.64-1.84; P = 0.75), bilirubin = OR, 0.98, CI, 0.90-1.07; P = 0.70), GGT = OR, 1.10, CI, 0.98-1.23; P = 0.09). No significant association was also observed between the genetic predisposition to T2DM risk and liver biomarkers. MR-Egger analysis showed no evidence of horizontal pleiotropy in the instrumental variables (IV) or weak instrument bias (Fst 10 for all the IVs). Whilst evidence of association was not found between genetically proxied liver biomarkers and T2DM risk and vice versa, our result showed consistency with observational studies that suggest there is a linear relationship between liver biomarkers and T2DM risk. Sample size may have been a limitation in this study, our results are however consistent with other MR studies that found no significant associations between some liver biomarkers and T2DM risk

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COMPUTATIONAL BIOLOGY AND AI

LINKING GENOMIC AND GENETIC DATA TO PROTEINS IN UNIPROTKB

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UniProtKB/Swiss-Prot is a reference resource of manually reviewed protein sequences enriched with expert curated information on protein functions, interactions, sequence domains, post-translational modifications, genetic variation, and disease. Information is extracted from peer-reviewed scientific publications and synthesized in gene-centric protein entries by professional curators in a way that it is Findable, Accessible, Interoperable and Researchable (FAIR). In this context, we report the effects of in vitro mutagenesis, as well as, for human proteins, the characterization of genetic variants observed in vivo. We classify natural variants associated with diseases following the ACMG guidelines, and our functional annotations provide the evidence required for the proper usage of the ACMG functional criteria. We also submit variant classifications to ClinVar, where the ACMG criteria used are reported. Currently, more than 86’000 variants are catalogued in the database, 40’000 of them are linked to genetic diseases, and over 10’000 are enriched with functional data. Taken together, this work will increase the coverage and usability of curated variant data in UniProtKB, and the utility of UniProt as a platform to integrate genome and variation data with the knowledge of protein function and disease.

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CANCER GENOMICS

EVALUATION OF HEREDITARY BREAST CANCER PATIENTS WITH AN EXPANDED GENETIC PERSPECTIVE: BRCA AND NON-BRCA GENES

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Breast cancer (BC), the most prevalent cancer worldwide, holds the highest mortality rates. Familial breast cancers constitute 5-10% of all breast cancers, with only 20-25% attributed to pathogenic variants in the BRCA1/BRCA2 genes. Additionally, other susceptibility genes, including TP53, NGO11, PTEN, ATM, PALB2, and CHEK2, have been identified, and an increasing number of susceptibility genes are continually recognized. A study reported the frequency of BRCA1/BRCA2-associated breast and ovarian cancers in Turkiye as 10,8%, and variants in other genes in BRCA-negative BC is 7,8. But we are still far from knowing the true prevalence of other genes. In this study, our aim is to report the pathogenic/likely pathogenic (P/LP) variants detected in patients with BC at the Medical Genetics Department of Çanakkale Onsekiz Mart University.

Between 2020-2023, our department conducted next-generation sequencing on 99 patients diagnosed with BC. A 160-gene panel was utilized for 30 patients, while a 61-gene panel was applied to the remaining 69 patients.

Among the 99 patients analyzed, 6% (6/99) exhibited pathogenic/likely pathogenic (P/LP) variants in BRCA1, and 2% (2/99) in BRCA2. Additionally, P/LP variants in genes other than BRCA1/BRCA2 were identified in 11% (11/99) of the patients. These genes include CHEK2, ATM, RAD51C, BRIP1, MSH2, FANCA, FANCF, MUTYH, POLG, and RINT1. When evaluated with the variants of uncertain significance (VUS), we detected 41 genes associated with the susceptibility of BC. Of the 19 patients with detected P/LP variants, 12 were identified through the 61-gene panel (12/69), and 7 through the 160-gene panel (7/30). While there is no significant difference in the detection rates between these two panels, we anticipate that with the expanding list of genes associated with familial BC, the necessity for larger panels in patient analyses will grow.

The term BRCAx is used to describe familial BC cases found to be negative for these BRCA1/BRCA2. Although other susceptibility genes have been identified, they still do not explain the majority of cases. This study emphasizes the significance of broader screening for BC susceptibility genes. Conducting a similar study in a larger cohort will enhance our understanding of genetic factors in BC.

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RARE DISEASES

RARE AND VERY RARE DISEASES DIAGNOSED THROUGH NEXT-GENERATION SEQUENCING AT ÇANAKKALE ONSEKIZ MART UNIVERSITY MEDICAL GENETICS (2020-2023)

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Diseases occurring in less than 1 in 2000 individuals are defined as rare diseases. There are over 6000 different rare diseases, affecting approximately 6-10% of the world`s population, with 75 percent having a genetic origin. 77.3-80.7% of people with rare diseases are attributable to diseases in the highest prevalence range (1-5 per 10,000). Çanakkale is a small city at west end of Anatolia with 100.000 people in city centre and 540 thousand people interland; and very low frequency of consanguineus marriage. The aim is present rare diseases detected by next-generation sequencing methods at Medical Genetics Department of Çanakkale Onsekiz Mart University, between 2020-2023.

Between 2020 and 2023, 182 patients were diagnosed as rare diseases in through NGS (WES-Whole Exome Sequence, CES-Clinic Exome Sequence) analysis. Sixteen different diseases rarer than 1/1,000,000 were identified in 19 of these patients. These extra rare diseases include IDDDFP (Intellectual Developmental Disorder With Dysmorphic Faces And Ptosis)(MIM617333) in BRPF1 gene, Alexander Disease(MIM203450) in GFAP gene, Mowat-Wilson Syndrome(MIM 235730) in ZEB2, Escobar Syndrome(MIM265000) in CHRNG gene, KBG Syndrome(MIM 148050) in ANKRD11 gene, Kindler Syndrome(MIM173650) in FERMT1 gene, Intellectual Developmental Disorder Autosomal Recessive 3(MIM608443) in CC2D1A gene, Usmanı-Riazuddin Syndrome(MIM 619467, 619548) in AP1G1 gene, CEBALID Syndrome(MIM618774) in MN1 gene, Desmosterolosis(MIM602398) in DHCR24 gene, Coproporphyria(MIM 121300) in CPOX gene, Coffin-Siris Syndrome(MIM135900) in ARID1B gene, Renpenning Syndrome(MIM309500) in PQBP1 gene, Glass Syndrome(MIM 612313) in SATB2 gene, Neurodevelopmental Disorder With Impaired Speech And Hyperkinetic Movements(MIM618425) in ZNF142 gene, Snijders Blok-Campeau Syndrome(MIM618205) in CHD3. Except for Intellectual Developmental Disorder in the CC2D1A gene and IDDDFP, other extremely rare diseases were detected in one patient each. IDDDFP was identified in one family (Proband, proband`s mother, and proband`s sister), and Intellectual Developmental Disorder OR3(MRT3) with the CC2D1A mutation was detected in two siblings.

It is a fact that the widespread use of NGS methods facilitates the diagnosis of rare diseases. Even in small-populated cities like Çanakkale, detecting numerous very rare diseases suggests that these conditions may not be as rare as we once thought, highlighting the impact of the proliferation of NGS methods.

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SINGLE-CELL GENOMICS / SPATIAL GENOMICS

NOVEL CILIARY PROTEIN TRIM8 IS A MULTIFUNCTIONAL WORKHORSE DURING MITOSIS

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European Union's Horizon 2020 TRIM-NET Innovative Training Network (ITN) of Marie Sklodowska-Curie Actions (MSCA), Department of Life Sciences, University of Trieste, Italy

TRIM8 is an E3 ubiquitin ligase that functions as both tumour suppressor and oncoprotein. Earlier, we reported that TRIM8 interacts with key regulators of mitotic spindle assembly, and that TRIM8-knockdown results in mitotic delay and aneuploidy. In this study, we optimise a single-cell RNA sequencing (scRNA-seq) analysis pipeline to evaluate the impact of TRIM8-silencing in a mitotic stage-specific manner. With the aid of differential transcriptomic (scRNA-seq) and proteomic (liquid chromatography-tandem mass spectrometry or LC-MS/MS) approaches we show that depletion of TRIM8 perturbs the canonical "Cell Cycle Control of Chromosomal Replication" pathway and demonstrate that TRIM8 negatively regulates the expression of DNA topoisomerase 2-alpha (TOP2A), known to be essential for genomic integrity. We also show that TRIM8 downregulation induces substantial alterations in translation activity of cells and results in upregulation of polysome-bound MALAT1 mRNAs by means of significant changes in polysome profiling coupled with RNA-sequencing. Finally, our study reveals endogenous TRIM8 as a novel ciliary protein that that exerts post-translational regulation on the centrosomal protein CEP170, required for ciliary function and co-localises with it in the centrosomal region during all mitotic phases. Our study shows the dynamic role played by a TRIM family protein across various stages of mitosis for the first time, laying the foundation for exploring the therapeutic potential of TRIM8 in addressing cell cycle-related diseases, including cancer.

GENETICS OF COMPLEX DISEASES

OLIGOGENIC INHERITANCE OF RARE VARIANTS FOR AUTISM SPECTRUM DISORDER

Oligogenic Inheritance of Rare Variants for Autism Hyeji Lee[1,2], Ganghee Lee[1,2], Da Yea Song[3,4], Jung Woo Park[5], Jae Hyun Han[3,6], Jeewon Lee[7], Heejung Byun[8], Ji Hyun Son[8], Ye Rim Kim[3,4], Yoojeong Lee[3], Junehawk Lee[5], Jeong Ho Lee[9], Heejeong Yoo[3,4], Joon-Yong An[1,2,10]

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Autism spectrum disorder is a genetic disorder with high genetic and phenotypic heterogeneity, and various types of genetic factors have been identified as causes of autism. While the role of de novo variants and common variants in autism is evident, the impact of rare variants remains less clear. We hypothesized that the influence of these rare variants could be better understood through an oligogenic model, which considers the combined effect of a few variants on the phenotype. In our study, we analyzed whole genome/exome sequencing data from ~ 2,500 samples from Korean families with autism cases, and families of European ancestry from the Simons Simplex Collection and Simons Foundation Powering Autism Research initiative. We identified high-quality de novo variants, rare variants (allele frequency 0.1%) and common variants (allele frequency 5%) to assess their genetic association with autism. We observed a significant enrichment of both de novo protein-truncating variants and the polygenic score for autism cases in both Korean families and European ancestry families. To explore the oligogenic inheritance pattern of rare variants, we defined disruptive rare variants as all protein-truncating variants and damaging missense variants using 10 pathogenicity prediction scores. We then discovered gene pairs affected by the oligogenic inheritance of disrupted rare variants, which were significantly associated with autism cases. We investigated biological functions and developmental impacts of these gene pairs using published single-cell RNA datasets of the human brain tissue. We also examined their association with autism-related clinical phenotypes. While the specific gene pairs identified in Korean families and European ancestry families differed, they shared similar patterns in cell-type enrichment and phenotype association. We noted that these results were distinct cellular and phenotypic features from previously known autism genes enriched in de novo variants. To sum up, our research identified the oligogenic inheritance of rare variants in autism across diverse ancestries and delved into their biological and clinical characteristics.

* The authors declare that there are no financial or commercial conflicts of interest regarding the content of this abstract.

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GENETICS OF COMPLEX DISEASES

Do ATXN-1 intermediate poly-CAG expansions play a prognostic role in Amyotrophic Lateral Sclerosis?

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Repeat expansions involving the ATXN-1 and ATXN-2 genes have been shown to be part of the complex genetic architecture of amyotrophic lateral sclerosis. In 2012, the association between ATXN1 intermediate repeat lengths and ALS was first described by our research group. Since then, other studies have confirmed this association in other populations as well. In addition, we reported the co-occurrence of SCA1 and ALS in a large SCA1 pedigree and, further investigated the association between ATXN1 and the C9orf72 repeat expansions and showed that ATXN1 is a disease modifier that predisposes C9orf72 repeat expansion carriers to develop ALS.

In this study, we analyzed the frequency and clinical characteristics of 328 ALS patients with ATXN1 intermediate repeat lengths (coding for 32-33 glutamines, polyQ) in a cohort of Italian ALS patients. All the ALS patients were not carrying C9orf72, SOD1, TARDBP and FUS mutations.

Patients with an increased number of polyQ repeats ≥ 32 had a trend of survival shorter than those with

In our study, ALS patients with intermediate polyQ ≥32 ATXN-1 repeat show a non-significant but suggestive trend toward faster disease progression and shorter survival. These findings should be considered preliminary, and will need to be confirmed in future multicenter studies in a larger population. If confirmed, this finding could pave the way for refining individual prognostic predictions and improving ALS clinical trial design, as is already happening with ATXN-2.

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CANCER GENOMICS

HOST SOMATIC VARIATION IN HIV/HPV CO-INFECTED WOMEN WITH CERVICAL INTRAEPITHELIAL LESIONS (CIN3)

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Despite antiretroviral therapy use, HIV-positive women still face a six-fold increased risk of cervical cancer (CC). HIV-HPV co-infection has been linked to accelerated CC development. However, there are limited studies on the role of host somatic variations in HIV-positive and HIV-negative women on CC. Understanding these variations can help identify potential genetic factors contributing to accelerated CC development. This knowledge is important in targeting interventions and improving outcomes for HIV-positive women with CC. Therefore, this study aimed to investigate host somatic genetic variation between cervical biopsies obtained from HIV-positive women and comparing them to HIV-negative women.

The cases and controls were carefully age-matched. Archived cervical biopsies from 88 women (44 HIV-positive, 44 HIV-negative) attending Groote Schuur Hospital Cancer Clinic between 2020 and 2022 were used. HPV infection and type were confirmed using the Anyplex™II HPV28 Detection kit. In CC, six hotspot regions in the four commonly mutated genes (TP53, PIK3CA, PTEN, and EGFR) were genotyped using Polymerase Chain Reaction and validated using Sanger Sequencing. Missense variant pathogenicity was assessed using SIFT, Polyphen-2, and ClinVar tools.

HIV-positive women had a median age of [37(IQR:34-41)] and HIV-negative women [35(IQR:32-43)]. There was statistical significance; more HIV-negative women reported a family history of cancer (p=0.014), a history of tobacco smoking (p0.0001), menstruation irregularities (p=0.01), and use of contraception (p=0.023) compared to HIV-positive women. Common HPV types identified were HPV 16(58%), 35(16%), and 58(14%). A total of 232 genetic variants were identified, with HIV-positive women having a higher burden of pathogenic variants (31%) compared to 15% among the HIV-negative. Identified mutations included stop-gain, missense, synonymous, and intron variants. The genes, TP53 and PIK3CA had more stop-gain variants among HIV-positive women (4/5) compared to HIV-negative women with 1/5 of the 5 mutations. These damaging variants were more prevalent in women under 50 in both cohorts.

In conclusion, younger women (50 years) showed predominantly damaging variants, indicating more aggressive cancer, a possible reason for early onset in the younger cohort. HIV-positive women displayed a higher mutation burden in PIK3CA and pathogenic variants in TP53, emphasizing the need to further explore these genes in gene expression studies.

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SINGLE-CELL GENOMICS / SPATIAL GENOMICS

Patient avatars: how to cost-efficiently leverage hIPSCs to model human diseases

Patient avatars: how to cost-efficiently leverage Rosa De Santis[1,2], Francesco Panariello[2], Antonio Grimaldi[2], Onelia Gagliano[3,4], Silvia Angiolillo[3,4], Nicola Elvassore[3,4], Davidee Cacchiarelli[2,5]

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Human induced pluripotent stem cells (hiPSCs) can differentiate into any cell type of the human body, rendering them a valuable resource in personalized and regenerative medicine. Using hiPSC-derived organoids holds great promise for disease modeling and potentially evaluating drug responses at the organ level rather than the cell level.

Their differentiation is often hampered by high costs and intensive workloads. In addition, the expansion of hiPSCs is needed to allow their usage and may cause cytogenetic instability and accumulation of mutations.

To this end, we applied a highly efficient microfluidics protocol that enables the reprogramming of human somatic cells to hiPSCs in 15 d, which are subsequently self-assembled in epiblast cysts and then differentiated into organoids. This novel continuous three-dimensional process offers a fast-track approach to cost-effectively generating functional organoids, avoiding expansion.

Here, we aim to confirm the stemness maintenance during the reprogramming process and the subsequent differentiation potential from hiPSCs to organoids. To achieve this, we performed a multi-omics analysis, profiling scRNA and scATAC on pooled hiPSCs at different stages, to demonstrate the establishment of full pluripotency capability right after the reprogramming. This integrated approach allows us to characterize gene expression patterns and chromatin accessibility, enabling the construction of a gene regulatory network (GRN) of transcription factors (TFs) associated with pluripotency.

Our findings reveal dynamic expression patterns of TFs throughout different stages of pluripotency, validating that early hIPSCs possess pluripotent properties for successful organoid differentiation. This approach makes feasible the development of a cost-efficient methodology for generating patient-specific avatars and could have a significant potential for personalized medicine, revolutionizing drug testing, disease modeling, and treatment.

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RARE DISEASES

MODELING MYOTONIC DYSTROPHY TYPE 2 IN HUMAN CEREBRAL ORGANOIDS

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Myotonic dystrophy type 2 (DM2) is a multisystemic autosomal-dominant disorder characterized by proximal muscle weakness, myotonia, cataracts and cardiac conduction abnormalities. Although there is also clear evidence of central nervous system (CNS) dysfunctions in patients, the mechanisms causing cognitive and neurodevelopmental impairment are still poorly understood. In this study, we developed 3D human cerebral organoids (hCOs) derived from DM2 induced pluripotent stem cell (hiPSC) to recapitulate the earliest stages of brain development in patients. We are able to characterize ventricular, subventricular and forebrain zones both in DM2 and control (CTR)-hCOs, modeling 3D brain organization and functional network complexity. Moreover, DM2-hCOs show the main disease hallmarks, including the maintenance of the CCTG expansion and the presence of CCUG-containing ribonuclear foci. We then performed RNA-seq analysis to identify transcripts aberrantly expressed in DM2 vs. CTR-hCOs. A total of 333 genes are discovered to be differentially expressed between DM2 and CTR-hCOs, with 234 and 99 genes up and down regulated, respectively (padj 0.01 and |log2 fold change| 1). Interestingly, among the genes strongly downregulated in DM2-hCOs we find the neuron specific transcription factors TBR1 (T-Box Brain Transcription Factor 1), TBR2 (T-Box Brain Protein 2) and FOXG1 (Forkhead Box G1). Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA) confirm that in DM2-hCOs several biological processes related to nervous system development, as well as to synaptic and postsynaptic transmission, are enriched. Since alternative splicing contributes to DM2 pathogenetic mechanism and directly correlates with common symptoms of the disease, we are also investigating splicing alterations in our DM2-hCOs model using Differential Exon Usage (DEU) analysis. Our analysis of preliminary data reveals differentially Exon Skipping (ES) events in genes encoding for ion channels, synaptic scaffold proteins and neurotransmitter receptors, whose validation is still in progress. The characterization of DM2 hCOs will provide deeper insights into the molecular patho-mechanisms occurring during brain development, thus opening new perspectives for the development of cell-based therapies and drug screening not only for DM2 but also for other neurodegenerative diseases.

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NON-CODING RNA GENES

The natural antisense RNA of NFYC is overexpressed pancancer and controls cell cycle progression through in cis and in trans modes of action

Cecilia Pandini[1], Giulia Pagani[1], Martina Tassinari[1], Emanuele Vitale[2], Eugenia Bezzecchi[1], Mona Kamal Saadeldin[1], Valentina Doldi[3], Giuliana Giannuzzi[1], Roberto Mantovani[1], Matteo Chiara[1], Alessia Ciarrocchi[2], **Prof Paolo Gandellini**[1]

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Antisense RNAs (asRNAs) represent an underappreciated yet crucial layer of gene expression regulation. Generally thought to modulate their sense genes in cis through sequence complementarity or their act of transcription, asRNAs can also regulate different molecular targets in trans, in the nucleus or in the cytoplasm. Here, we performed an in-depth molecular characterization of NFYC Antisense 1 (NFYC-AS1), the asRNA transcribed head-to-head to NFYC subunit of the proliferation-associated NF-Y transcription factor.

Our results show that NFYC-AS1 is a prevalently nuclear asRNA peaking early in the cell cycle. Refined re-annotation of its transcript by in silico (interrogation of CAGE, 3'-seq data) and experimental approaches (5' and 3'RACE) revealed a main long isoform. NFYC-AS1 is overexpressed pancancer, preferentially in association with RB1 mutations. Functional analysis integrating guilt-by-association approaches and knockdown by two complementary methods, namely gapmer antisense oligonucleotides and CRISPR-Cas9 editing of the transcription start site (used to assess RNA- and transcription-dependent functions), revealed a role in supporting proliferation and mitotic progression in both RB1 wild type and mutated cancer cells (from squamous cell carcinoma and small cell carcinoma of the lung, respectively). However, the different silencing approaches revealed a dual mode of action, in cis and in trans. The in cis function appears to be transcription-dependent and is mediated by a transcriptional interference mechanism impinging on the sense NFYC gene, as supported by eQTL analysis and cell cycle re-entry experiments. Such mechanism is likely needed to finely tune NFYC transcription at the beginning of the cell cycle. It evolved and became predominant in mammals as compared to the more ancestral and less regulated NFYC downstream transcriptional start site. The in transfunction is instead RNA-dependent and at least in part NFYC-independent and impinges on the regulation of G2/M-specific genes, in line with the mitotic arrest phenotype arising from NFYC-AS1 silencing.

Overall, NFYC-AS1 emerged as a cell cycle-regulating asRNA with dual mode of action, as well as an optimal candidate to be evaluated in the long term as a new target entity for the development of novel personalized anticancer therapeutic approaches, including the treatment of the very aggressive RB1-mutated tumors.

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RARE DISEASES

CLINICAL EXOME REVEALS A NOVEL VARIANT IN WNT10A GENE IN A FAMILY WITH ECTODERMAL DYSPLASIA

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A 41 years-old male was addressed to genetic counselling for a suspected Hypohidrotic Ectodermal Dysplasia (HED) presenting anomalies of teeth, hair (hypotrichosis) and nails with bone fragility; his father presented a nonsyndromic teeth anomaly, while the mother is apparently healthy.

Since previous Next Generation Sequencing (NGS) analysis of EDA gene and gene-targeted deletion/duplication analysis using SALSA MLPA (Probemix P183) assay including EDA, EDAR, EDARADD, WNT10A genes yielded negative results, we applied a clinical exome sequencing (CES) on the proband's and both parents gDNA, using the Clinex Pro (4Bases) kit according to the manufacturer`s protocol. The CES was performed using Ion S5 Prime (Thermofisher) and data were analyzed with the Geneyx Analysis software.

CES in the proband reveals a heterozygous variant NM_025216.3:c.286TC, p.(Cys96Arg) in exon 2 of WNT10A gene inherited from the father and a heterozygous variant NM_022363.4:c.68CT, p.(Ser23Leu) in exon 3 of EDAR gene inherited from the mother. The variant c.286TC is classified as Variant of Unknown Significance (VUS), while the c.68CT variant is reported on ClinVar as Conflicting interpretations of pathogenicity between VUS and Likely benign and segregated in his healthy sister.

Homozygosity or compound heterozygosity for variants in WNT10A gene are associated with Schopf-Schulz-Passarge syndrome (AR), Ectodermal dysplasia 16 (AR), and Tooth agenesis (AD/AR). In the families with isolated tooth agenesis, heterozygous subjects present variable hypodontia with/without other manifestations of ectodermal dysplasia, suggesting the high degree of variability in phenotypic expression.

Our clinical and genetics findings confirm the difficulty to define a recognizable genotype/phenotype correlation with an impact on genetic counselling issues in providing a correct clinical and molecular diagnosis.

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NON-CODING RNA GENES

THE INTERPLAY BETWEEN ALTERNATIVE POLYADENYLATION AND MICRORNAS IN THE REGULATION OF ONCOGENIC TRANSCRIPTION FACTORS

Giulia Pagani[1], Stefania Fornezza[1], Chiara Casirati[1], Cecilia Pandini[1], Paolo Gandellini[1]
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Most human genes undergo alternative cleavage and polyadenylation (APA), which regulates 3'UTR length and accessibility. APA ultimately affects mRNA stability and protein level by altering microRNA (miRNA) and RNA-binding protein (RBP) activity. NFYA is an oncogene encoding for the regulatory subunit of the nuclear transcription factor Y, which controls key transcriptional changes in pathways broadly deregulated in cancer. NFYA is strongly overexpressed in human tumors and the poor correlation between NF-YA protein and mRNA in different cancer types suggests that APA and miRNA regulation may be as relevant as other mechanisms to control NF-YA activity in cancer. Concerning this, we employed APAtrap tool, together with 3' end sequencing data (from polyADB and polyAsite), to correctly re-annotate the NFYA 3'UTR repertoire. Such analysis identified multiple APA sites resulting in four possible NFYA 3'UTRs, all supported by the presence of the canonical polyadenylation signal (PAS). We observed that tumor and immortalized cells mainly use shorter 3'UTRs compared with normal cells, which prevalently use longer ones. In this regard, we found an increased NF-YA protein/mRNA ratio to be associated with higher usage of shorter 3'UTRs across 233 cancer cell lines. Moreover, the effect of different NFYA 3'UTRs on transcript stability, protein level, and miRNA/RBP binding is under study through the use of different strategies to alter NFYA 3'UTR usage. We experimentally validated that increased usage of shorter NFYA 3'UTRs, induced either exogenously or endogenously, is associated with higher NF-YA protein/mRNA ratio in prostate adenocarcinoma cells. As expected, 3'UTR shortening also leads to relief from regulation by miRNAs having distal binding sites. Recently, we obtained genomically deleted clones lacking the PAS of the most abundant NFYA 3'UTR isoform. The characterization of such clones is ongoing to assess the molecular and biological implications of such deletion.
Overall, APA and miRNA regulation appear relevant post-transcriptional mechanisms that control NF-YA activity in cancer. Notably, the emerging application of ASOs in the clinics opens the possibility to target/mask PAS or miRNA/RBP binding sites to therapeutically manipulate the effects of APA and miRNAs in cancer.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

HEREDITARY PANCREATITIS DUE TO MISSENSE VARIANT OF THE PRSS1 GENE

HEREDITARY PANCREATITIS DUE TO MISSENSE VARIANT OF Adriana Carolina Rubio Roa[1], Juan Diego García Ortiz[2], Orietta Ivonne Beltrán Casas[3,4]
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INTRODUCTION:

Hereditary pancreatitis (HP) (MIM 167800) is a rare disease, with autosomal dominant inheritance and locus heterogeneity that often presents in early childhood and can induce multiple episodes of acute and chronic pancreatitis, gradual loss of pancreatic parenchyma, higher rates of exocrine failure and diabetes over time. Different genetic variants cause a disequilibrium between secretion of proteolytic enzymes and the regulatory proteins that act as inhibitors, leading to autodigestion of the pancreatic parenchyma.

We report a case of a 9 year-old boy with abdominal distension, meteorism and poor weight gain in whom a genetic variant in the PRSS1 gene was found.

OBJECTIVES: To analyze the clinical case of a pediatric patient with intestinal malabsorption of unknown cause.

RESULTS:
A 9-year-old child with a 5 year medical history of meteorism, abdominal pain and poor weight gain. Healthy and controlled pregnancy, non-consanguineous parents with no episodes of pancreatitis. Alimentary allergies were excluded. The patient had no symptom resolution with dietary adjustment. In the suspect of cystic fibrosis vs. pancreatic insufficiency a multigenic panel study for the genes CTRC, SPNK1, CFTR, PRSS1, PRSS2 revealed a probable pathogenic heterozygous variant in PRSS1 c.41CT (p.Ala16Val). Pancreatic enzymes replacement were initiated with important clinical improvement.

CONCLUSION: In the approach to intestinal malabsorption, gastrointestinal and respiratory symptoms should be taken into consideration given the high frequency of cystic fibrosis (prevalence of 1: 10.000). However, cases without systemic signs and symptoms suggest more to a primary gastrointestinal pathology and/or its accessory glands. In HP, establishing a timely management enables a reduction of pancreatic tissue destruction and thus reduces the risk of developing pancreatic cancer and diabetes in adulthood. Additionally, HP shows variable expressivity and incomplete penetrance, with the onset of symptoms in childhood or adolescence, therefore, in genetic counseling, the absence of a family history does not rule out vertical transmission.

POSTER SESSION 2
APRIL 09
FROM 10:15 TO 11:00

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REPRODUCTIVE GENETICS

WHOLE GENOME SEQUENCING IDENTIFIES POTENTIALLY DELETERIOUS GENETIC VARIANTS IN RECURRENT MISCARRIAGE CASES IN MALAYSIA

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Human reproduction is highly inefficient, and it is estimated that only 30% of all human conceptions result in live birth. Recurrent miscarriages, which affect 2-4% of women, is defined as the loss of two or more pregnancies within 24 weeks of gestation. The most common genetic cause of miscarriage is chromosomal aneuploidy, but a large proportion of recurrent miscarriages that are classified as idiopathic, may also occur due to small (50 bp) changes in the genomic sequence. In this study we carried out a systematic and comprehensive mutational analysis of the miscarried foetus and its biological parents using short-read whole genome sequencing on an Illumina platform. We sequenced 5 euploid miscarried foetuses and their biological parents utilized a robust bioinformatics pipeline to prioritize 567 rare (0.01 frequency) variants in 496 genes coding for 1,285 transcripts. Only 13/567 of these were predicted to have a high impact and severe consequences possibly causing the recurrent miscarriage. In each sample we identified unique variations in developmental genes implicated in brain development or embryonic lethality in knock-out mouse models. The highlighted genes included reelin (RELN) and EPH receptor B1 (EPHB1) involved in brain development, and histone deacetylase 6 (HDAC6), insulin like growth factor 2 receptor (IGF2R) and transmembrane 131 like (TMEM131L) associated with embryonic or perinatal lethality in mice.

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RARE DISEASES

RNA-BASED DOWNREGULATION OF PHOX2B EXPRESSION AS A PUTATIVE CCHS THERAPEUTIC

RNA-Based Downregulation of PHOX2B Expression as a Buwei Shao[1], Daniel Falik[2], Ori Kahiri[2], Gad Vatine[2], Avital Adato[3,4], Karen Avraham[1]

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare life-threatening neurological disorder that is characterized by compromised chemosensitivity and inadequate autonomic control of breathing. The cause is mainly attributed to de novo heterozygous polyalanine repeat expansion mutations (PARMs) in Paired-like homeobox 2b (PHOX2B). PHOX2B is a gene that plays a key role in regulating the development of the autonomic nervous system. So far, there are no therapies available for treating CCHS. A recent study, conducted on patient-derived autonomic neurons, revealed that PARMs induce a toxic gain of function, leading to cell death among PHOX2B-expressing cells. In addition, clinical observations have reported that two CCHS patients, utilizing the synthesized progestin as a contraceptive, exhibited amelioration of respiratory symptoms. Subsequent studies showed that this synthetic progestin downregulate PHOX2B expression both in cell and rat models. Together, these studies suggest that reducing PHOX2B expression could be clinically beneficial for CCHS patients. In this study, our aim is to develop antisense oligonucleotides (ASOs) to downregulate PHOX2B expression that might alleviate the respiratory symptoms in CCHS patients.

We designed and prioritized 172 chimaeric 2′-O-methoxyethyl/DNA gapmer ASOs that target human PHOX2B mRNA. Five out of 18 ASOs that were screened in a HEK293T cell model showed significant inhibition of PHOX2B expression. To further explore their clinical relevance, lead ASOs are being tested in disease iPSC-derived autonomic neurons that express PHOX2B endogenously and in mice models. Our research has the potential to advance the development of innovative therapeutic strategies for CCHS, effectively alleviate symptoms and improve disease management.

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GENETICS OF COMPLEX DISEASES

Genetic characterization of a cohort of patients with severe atopy suspicious for Inborn Errors of Immunity

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Background

Severe allergic dysregulation is described in many classical Inborn Errors of Immunity (IEIs) and sometimes may be the initial presentation of the immune dysregulation.[1] Among IEIs, Primary Atopic Disorders (PADs) present with dysregulated pathogenic allergic effector responses. To date, at least 40 monogenic disorders can be subcategorized as PADs.[2] Affected patients are often refractory to standard therapies and require alternative therapeutic options.[3]

Methods

We describe a pediatric cohort of patients with severe atopic manifestations (i.e., early onset, severe, refractory to standard therapies atopic dermatitis [AD], asthma, food allergies with or without anaphylactic shock) and suspicious for IEI. Next generation sequencing (panels, WES, WGS) have been performed.

Results

We enrolled 20 patients (n=13 females n=7 males) between 2019 to 2023, among 2000 allergic patients. The predominant clinical manifestation was severe early-onset AD (89%), followed by respiratory allergies (72%) and food allergies (67%), associated in 19/20 patients to high IgE levels (range 319-41500 kU/L). Median age at symptoms onset was 2 months. In 9 patients (45%) definitive genetic diagnosis was made at a median age 7 years. Two patients had Hyper IgE Syndrome (HIES) due to STAT3-LOF (loss-of-function) mutation. Two patients were diagnosed with Combined Immunodeficiency (CID): P4 with DOCK8 deficiency and P5 with ARPC1B deficiency. P9 had HIES-CADINS due to CARD11-LOF mutation. P10 showed FLG deficiency, P19 had DSP and FLG deficiency. One patient showed a STAT6-GOF (gain-of-function) mutation that identifies a new primary atopic disorder.[4] In some cases, WGS or functional analysis were needed for the diagnosis. In 10 patients no clear causative mutations have been identified but we detected heterozygous variants of uncertain significance (VUS) in IEI genes associated (i.e., ZNF341, SPINK5, ADA, AIRE, CARD11).

Conclusion

Severe early onset refractory atopic diseases could represent clinical warning signs of an underlying IEI. Molecular diagnosis is really helpful to clarify the pathogenesis. Variants' interpretation is always challenging, and deeper investigations may be useful to validate the role of VUS in these patients, as well as sharing clinical and genetic data with other groups.

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RARE DISEASES

COQ7 DEFECT CAUSES PRE-NATAL ONSET OF CARDIOMYOPATHY AND GASTROINTESTINAL OBSTRUCTION IN A FAMILY WITH MITOCHONDRIAL COQ10 DEFICIENCY

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Recessive bi-allelic variants in the COQ7 gene cause primary Coenzyme Q 10 (CoQ10) deficiency associated with three main clinical phenotypes based on our recent literature review: 1) encephalopathy, characterized by an ataxic gait, spasticity, central hypotonia, and sensory visual/hearing impairment; 2) peripheral neuropathy, presenting with axonal motor neuropathy impacting distal lower limbs with/without upper motor neuron involvement; and 3) neonatal-onset multisystemic disease with dysmorphisms and variable involvement of the kidney, brain, gastrointestinal tract, lungs, and heart.

Here, we present novel compound heterozygous mutations identified in the COQ7 gene, leading to the prenatal onset (20 weeks of gestation) of hypertrophic cardiomyopathy and intestinal dysmotility in a consanguineous family from Bangladesh, with both siblings being affected. The principal clinical findings included dysmorphisms, recurrent intestinal occlusions requiring ileostomy, left ventricular non-compaction cardiomyopathy, ascending aorta dilation, arterial hypertension, renal dysfunction, diffuse skin desquamation, axial hypotonia, neurodevelopmental delay, and growth retardation.

Exome sequencing disclosed compound heterozygous rare variants in the COQ7 gene, c.613_617delGCCGGinsCAT (p.Ala205HisfsTer48) and c.403AG (p.Met135Val). In silico analysis and functional in vitro studies confirmed the pathogenicity of the variants, responsible for abolished activities of complexes I+III and II+III in muscle homogenate, and a severe decrease of CoQ10 levels together with reduced basal and maximal respiration in patients` fibroblasts.

Although oral CoQ10 supplementation ameliorates psychomotor development in the first proband, she suddenly passed away at 14 months with multiorgan failure during an infective episode. However, supplementation with a high dose of CoQ10 (30 mg/kg/day) since the first days of life modified the clinical

course in the second child, showing the stabilization of renal and intestinal involvement and recovery of milestones acquisition at the last follow-up (18 months of age).

Our study expands the clinical spectrum of primary CoQ10 deficiency due to COQ7 gene defects and identifies antenatal clinical signs of the disease that can prompt early diagnosis and treatment supplementation with a positive outcome.

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PRECISION HEALTH

Delving into the role of the HLA status in patients with chronic spontaneous urticaria.

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Chronic spontaneous urticaria (CSU) is a dermatological disorder defined by the presence of urticaria on most days of the week for six weeks or longer. The condition affects 0.5%–1% of the general population and autoimmunity is hypothesized to be responsible for about one-third of CSU cases. A correlation between dermatological diseases with immune pathogenesis and HLA polymorphisms is documented in the literature. Recent studies support the association between the antigen expression of the major histocompatibility complex (MHC) and CSU predisposition as well.

In our retrospective study, HLA class I antigens were characterized by Sequence-Specific Oligonucleotide (SSO) assay on DNA extracted from peripheral blood samples of 20 patients with CSU.

Antigens frequencies (HLA-A, HLA-B, and HLA-C) from patients and a control group from the same geographic region (n=100) derived from the IMGT/HLA Database were compared by Fisher exact test. When HLA-A and HLA-B alleles were compared, no significant difference in distribution was reported in patient and control groups (p 0.05). Nonetheless, HLA-C05 allele frequency was statistically significant compared to the control group (p=0.016) with an OR value of 4.235 (1.382-12.975).

The role of the HLA I class in the process of autoimmunity is not fully clear. Specific HLA-A antigens have been recently associated with CSU protection in previous observational studies, yet a precise comparison was difficult due to ethnic differences. To our knowledge, we describe the first known association between HLA-C*05 allele and CSU predisposition; particularly in the Italian population. HLA-C genes encode molecules involved in presenting antigenic peptides, interacting with natural killer (NK) cell receptors, and inhibiting non-MHC-restricted T cells and NK.

The extreme variability determined both by HLA polymorphism and the diverse regional environmental factors could justify the heterogeneity of the results evidenced in different populations to date. Further studies, possibly prospective and with a larger cohort of CSU patients, will be necessary to demonstrate this observation. Nonetheless, these results further support a genetic basis in the CSU pathogenesis, involving interactions between HLA alleles.

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SINGLE-CELL GENOMICS / SPATIAL GENOMICS

SCALT: AUTOMATIC IDENTIFICATION OF CELL TYPES FROM SINGLE-CELL RNA SEQUENCING DATA

SCALT: automatic identification of cell types from Daniele Traversa[1], Matteo Chiara[1]
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Single cell RNA sequencing (scRNAseq) marks a key methodological breakthrough for the study of the organization and function of cells and cell types. The key concept behind scRNAseq is relatively simple: gene expression profiles are collected at single cell resolution by micro fluidic devices or equivalent methods; subsequently gene expression patterns are used as a proxy to define similar cell types and infer their identity.

Dimensionality reduction and unsupervised clustering currently represent the de facto standard methods for the analysis of scRNAseq data. These techniques however suffer from inherent limitations including: 1) need for manual/expert curated annotation of cell types; 2) general lack of reproducibility (since manual annotation); 3) limited resolution in the identification and annotation of scarcely represented cell types. As a result, current analytical workflows require extensive analyses and the application of different sets of parameters for an accurate delineation of cell type clusters and their annotation.

Here, we present SCALT (Single Cell Annotation Likelihood Tool), an innovative method which introduces a paradigm-shift for the analysis of scRNAseq data. In our approach, cells are annotated to a specific type at individual level, by using a simple but elegant method based on maximum likelihood, without the need for clustering, dimensionality reduction or manual annotation. SCALT leverages a collection of 471 lists of cell-type specific genes, constructed by extensive re-analysis of comprehensive and expert curated catalogues (HPA and DISCO).

Applied to the reference benchmark dataset by Abdelaal et. Al 2019, SCALT performed comparably or better than other the methods therein tested. Further, it recognized the correct cell type for 98.7% and 98.8% of the over 553411 and 4339209 distinct cells included in HPA and DISCO, respectively.

In conclusion, SCALT represents an innovative and highly useful method for the analysis of scRNAseq assays.

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RARE DISEASES

MUTANT SPARTIN IMPAIRS TRANSCRIPTION OF GENES INVOLVED IN BIOENERGETICS METABOLISM AND ALTERS THE MITOCHONDRIAL PROTEIN IMPORT

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Pathogenic variants in SPART gene cause Troyer syndrome, a recessive form of spastic paraplegia characterized by lower extremity spasticity and weakness, short stature and cognitive impairment.1,2,3

To identify the overall altered biological pathways due to loss of function mutations in Spartin, we performed whole RNA-sequencing in a neuronal-derived genome-edited cell line harboring a homozygous SPART variant that we previously identified via whole exome sequencing, SYc.892dupA.4

We identified 707 genes with differential expression between mutant vs. control cells at an adjusted Pvalue (padj)0.05. Gene ontology analysis revealed that the top significant downregulated biological pathways in mutant cells vs. controls were related to transcriptional and translational processes and, notably, to the oxidative phosphorylation metabolism.

We therefore performed functional studies to investigate the pathways involved in mitochondrial impairments using the SYc.892dupA cell model and skin-derived fibroblasts carrying biallelic variants in SPART. Both cell models exhibited an altered mitochondrial network, an increased mitochondrial oxidative stress and a decreased mitochondrial respiration with a reduced expression of the mitochondrial respiratory chain enzymes and a partial rearrangement of their assembly. We also found a disrupted calcium dynamics and a defective import of nuclear-encoded mitochondrial proteins, compared to control cells.

To demonstrate that mutant Spartin was the cause of the altered mitochondrial functionality, we evaluated the bioenergetics parameters in mutant cells after transient transfection with wild-type Spartin. In both cell models, wild-type Spartin re-expression led to increased ATP/ADP ratio comparable to control cells, with the recovery of intracellular free calcium levels and rescue of the import of nuclear-encoded mitochondrial proteins. In addition, transient transfection of wild-type Spartin restored the expression of several genes encoding for different subunits of the OXPHOS enzymes (i.e. MT-ND3, MT-CO3, MT-ATP6, TIMM13).

Taken together, these data suggest that Spartin modulates the mitochondrial functionality regulating the transcription of the genes involved in the energy production and in the import of nuclear-encoded mitochondrial proteins.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

COMPUTATIONAL ANALYSIS OF SNPS ASSOCIATED WITH CONGENITAL HEART DISEASE

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Congenital heart disease (CHD) encompasses structural heart defects present at birth, impacting the chambers, valves, and vessels of the heart. Genome-wide association studies (GWAS) have unveiled a significant relationship between single-nucleotide polymorphisms (SNPs) and their involvement in CHD pathogenesis. Intriguingly, over 90% of these SNPs are situated in the noncoding part of the genome, adding complexity to the comprehension of their mechanisms. We established a systematic computational pipeline to identify and analyze CHD-associated SNPs across both coding and noncoding regions of the genome. First, we compiled a thorough dataset of SNPs from the GWAS catalog and ClinVar database, filtering them based on CHD-associated traits. Subsequently, these CHD-SNPs were annotated and categorized into noncoding and coding regions based on their location. To investigate the impact of noncoding CHD-SNPs on gene regulatory mechanisms, we cross-validated them with enhancer-specific histone modification marks obtained from the developing human heart across 9 Carnegie stages and identified potential cardiac enhancers. This approach led to the identification of 2,056 CHD-associated putative enhancers (CHD-enhancers), with 38.9% exhibiting overlap with known enhancers cataloged in the human enhancer disease database. We identified heart-related transcription factor binding sites within these CHD-enhancers, providing insights into the influence of SNPs on transcription factor (TF) binding. Conservation analysis further unveiled that many of these CHD-enhancers exhibit a high degree of conservation across vertebrates, implying their evolutionary significance. Leveraging heart-specific expression quantitative trait loci data, we identified a subset of 63 CHD-SNPs with regulatory potential distributed across various cardiac tissues. Simultaneously, we represented coding CHD-SNPs as a protein interaction network and its subsequent binding energy analysis focused on a pair of proteins within this network, pinpointed a deleterious coding CHD-SNP, rs770030288, located in C2 domain of MYBPC3 protein. Taken together, our findings highlight that SNPs have the potential to impact gene regulatory systems, whether through modifying enhancer sequences or by disrupting protein-protein interactions, which can cause alterations in the early developmental processes and contribute to CHD pathogenesis.

PRECISION HEALTH

GENOME-SCALE DEPENDENCY MAPS REVEAL HINTS ABOUT THE FUNCTION AND THERAPEUTIC VALUE OF AN ONCOGENIC TRANSCRIPTION FACTOR IN HUMAN CANCER

From Genome-Scale to precision oncology: targeting Alessia Canevotti[1], Camilla Vitali[1], Giacomo Graziano[1], Alessandro Macchia[1], Giulia Pagani[1], Paolo Gandellini[1]
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The key transcription factor NFY is frequently overexpressed in human tumors and is comprised of three subunits: NF-YA, which binds to the CCAAT box, NF-YB and NF-YC. Its involvement in cancer is linked to its impact on genes associated with DNA replication, cell cycle progression, and metabolism. Particularly relevant is the elevated expression of NFYA regulatory subunit across diverse cancer types. Despite the indications suggesting that inhibiting NFY subunits may damage tumor cells, the therapeutic potential of NFYA targeting in human cancer remains largely unexplored.
In this study, we employed genome-scale cancer dependency maps to examine the vulnerability of a thousand cancer cell lines to NFYA inhibition obtained by either RNA interference (RNAi) or CRISPR/Cas9.
We found that NFYA is essential for 20% of cancer cell lines, which is a lower percentage as compared to well-established oncogenes like MYC and KRAS, to which almost all cell lines are dependent, but in line with that of other more selective factors, such as EGFR. Notably, most dependent cell lines were from lung and pancreatic cancers. The fitness effect of NFYA depletion, calculated individually from RNAi and CRISPR screening or as combined score, seemed to be unrelated to mRNA and protein expression levels or the copy number of any among NFY subunits. However, by investigating the genes correlated with NFYA scores, we could uncover genes involved in estrogen response as possible determinants of vulnerability. In addition, NFYA-dependency signature was related to a lower survival probability in lung adenocarcinoma, suggesting on one hand that most aggressive tumors could be particularly vulnerable to NFYA inhibition, and on the other that this signature may represent a predictive biomarker of tumor response to NFYA depletion.
Finally, by conducting correlation analyses between NFYA score and fitness scores for other genes (co-dependencies), we were able to reveal hints about NFYA function and identify novel putative interactors.
In a personalized medicine perspective, this research has the potential to explore the therapeutic value of NFYA inhibition in cancer and to reveal novel targetable factors, as well as to identify biomarkers able to predict response of individual patient’s tumors to NFYA depletion.

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CANCER GENOMICS

The Potential Role of the POLG Gene in Cancer: A Candidate for Hereditary Cancer Syndromes

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DNA polymerase γ (POLG) is the sole mitochondrial DNA polymerase and plays a role in the replication and repair of mitochondrial DNA (mtDNA). POLG has been associated with mitochondrial DNA depletion syndrome and neurological diseases in databases such as OMIM. In the Cancer Somatic Mutation (COSMIC) database, POLG has been categorized as a tier 2 gene. However, studies on its relationship with hereditary cancer syndromes are limited.

In most tumorogenesis studies, nuclear genes encoding tumor suppressors and proto-oncogenes have been investigated; conversely, nuclear genes responsible for mitochondrial proteins have been rarely evaluated. However, mitochondria are also crucial for cancer processes as they are the powerhouse of the cell. The role of POLG in mitochondrial DNA replication supports its role in cancer genetics.

The aim of this study is to contribute to the potential role of mutations in the POLG gene in hereditary cancer syndromes.

In our laboratory, Next Generation Sequencing (NGS) analysis was performed on individuals with a family and/or personal history of cancer. Commonly reported T251I and P587L mutations in the POLG gene were identified in 4 patients. No pathogenic or likely pathogenic variants were detected in other genes in these patients. The patients and their histories were as follows:

· Patient 1: A 29-year-old female diagnosed with breast cancer, with a family history of breast, lung cancer, and lymphoma.

· Patient 2: A 66-year-old female diagnosed with breast cancer two years ago, with a family history of breast, thyroid, lung cancer, and lymphoma.

· Patient 3: A 57-year-old female with a family history of large cell mediastinal lymphoma, breast, stomach, lung, and endometrial cancer.

· Patient 4: A 56-year-old female with a family history of endometrial and prostate cancer.

POLG represents a promising candidate in the genetic etiology of hereditary cancer syndromes. This relationship can be better understood with future functional studies. Furthermore, future studies evaluating somatic and germline mutations in mitochondrial biogenesis genes like POLG may lead to the development of targeted oncologic therapeutics, particularly utilizing metabolism pathways such as glycolysis and oxidative phosphorylation.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

RECLASSIFICATION OF A NOVEL SYNONYMOUS *PGAP1* VARIANT DISRUPTING SPLICING

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Variants of uncertain significance (VUS) represent a major challenge in clinical genetic testing and their impact is often underestimated. In particular, synonymous variants may affect splicing mechanisms causative of multiple monogenic disorders. *In-silico* tools are not always effective in predicting the impact of these variant and their characterization remains challenging.

In this study, we investigated a 14-years-old girl affected by global neurodevelopmental delay, absent speech, flaccid tetraparesis, and brain atrophy, born to healthy consanguineous parents. Trio whole-exome sequencing (WES) revealed in the proband the novel homozygous synonymous variant c.1272GA (p.Lys424Lys) in the *PGAP1* gene, inherited from the heterozygous parents. Despite its synonymous nature, this variant was initially classified as a variant of uncertain significance (VUS), as it is predicted by in-silico tools to alter the donor splice site of the *PGAP1* exon 12. Target mRNA sequencing confirmed the expression of a mutated isoform with skipping of exon 12 in the proband and the presence of both the wildtype and exon 12-skipped isoforms in her parents. The exon skipping induced by the identified mutation leads to a frameshift and the generation of a premature stop codon at the amino acid position 409, further resulting in nonsense mediated decay or the synthesis of a truncated protein that lacks 514 amino acids, including part of the catalytic domain and the two stabilizing jelly-roll domains. Biallelic *PGAP1* mutations are responsible for the autosomal recessive neurodevelopmental disorder with dysmorphic features, spasticity, and brain abnormalities (NEDDSBA; OMIM #615802), a syndrome strongly correlated with the proband's phenotype.

In conclusion, the characterization of this *PGAP1* transcript allowed the reclassification of this variant as likely pathogenic and highlights the importance of extending the analysis and the evaluation of synonymous variants in cases with a strong gene-disease correlation. The analysis of the variant identified in this family allowed to reach a conclusive diagnosis and to extend the phenotypic spectrum associated with NEDDSBA, since, to our knowledge, this is the first report flaccid tetraparesis in this disorder.

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NON-CODING RNA GENES

EXPANDING THE ANNOTATION OF NON-CODING RNAS IN THE GENCODE GENESET

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The Ensembl-HAVANA team produces comprehensive reference gene annotation for human and mouse genomes (GENCODE geneset). Producing accurate gene annotation is of fundamental importance for both genome biology and clinical genomics; incorrect or incomplete annotation can impact downstream analysis and introduce potentially significant errors. With the increased knowledge of the functional roles of non-coding genes, this principle becomes increasingly applicable beyond known protein-coding genes. The annotation of non-coding genes is less mature than that of protein-coding genes. Here we will present recent work in the expansion and refinement of our non-coding RNA catalog, of both long non-coding RNAs (lncRNAs) and small RNAs, supporting our aim to produce accurate and comprehensive reference annotation for all gene biotypes.

As part of the GENCODE consortium, we have expanded lncRNA annotation in both human and mouse genesets with the integration of capture long-read sequencing (CLS) data sets to add more than 100,000 novel lncRNA transcripts to the annotation. We will discuss the updates to lncRNA annotation and the challenges presented by the classification and visualisation of large numbers of new transcript models. We will also describe the first agreed set of lncRNA transcripts that we have investigated with the RefSeq team to add reference transcripts for non-coding genes of clinical relevance to the Matched Annotation from NCBI and EMBL-EBI (MANE) set.

With the increase of biological knowledge of small RNAs, we aim to evolve our GENCODE catalog of small RNA annotation to more accurately represent the transcription and processing of functional molecules. Therefore, we will outline how we are using external expert knowledge and our own manual and computational workflows to improve and expand our representation of small RNA biology in GENCODE.

Our annotation is accessible via Ensembl, the UCSC Genome Browser and https://www.gencodegenes.org.

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REPRODUCTIVE GENETICS

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 presence of small amounts of cell-free fetal DNA (cffDNA) circulating in maternal blood. Conventional 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 overcome these challenges and increase the accuracy, we developed a new NIPAT test (NEWPAT) based on double nucleotide polymorphisms (DNPs).

First, we identified 978 genome-wide DNPs, selected from the Genome Aggregation Database using several filtering criteria. We then performed a target enrichment of these markers using the Ion AmpliSeq™ Kit Plus from Thermo Fisher Scientific and sequenced them by NGS using the Ion Torrent™ S5 system. The approach was validated by: (a) sequencing 15 individuals from 4 families at a depth of 5000X, to assess Mendelism and DNP amplification bias; (b) re-analysing the same samples at lower depths (500-1000X) to evaluate sensibility; (c) sequencing at high depth (10,000X) 12 pregnant women with decreasing estimated proportions of cffDNA and 12 biological fathers of the fetuses (1000X) to assess system reliability and estimate combined paternity index (CPI) in real cases.

Since DNPs do not represent markers routinely used in most of the genomic analyses, a standard bioinformatic workflow to analyze them from NGS data has not been developed yet. So, we developed an ad hoc pipeline, applying rigorous quality control to ensure the accurate calling of alleles at each position of the DNPs and to check at the same time for contamination.

Our approach significantly improved the discrimination between cffDNA alleles and sequencing errors, which is essential for the accurate identification of paternal alleles. The sequencing error rate at most DNPs was close to zero and in any case orders of magnitude lower than commonly used SNPs, emphasizing the improved precision of our sequencing strategy. For all the pregnancies tested, the CPI was well above the threshold for paternity attribution.

This innovative method is crucial for the advancement of prenatal diagnostics as it offers a new level of precision and reliability in genetic testing.

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GENETICS OF COMPLEX DISEASES

ALTERATION OF TELOMERE LENGTH AND TERF1 GENE EXPRESSION LEVELS IN RHEUMATOID ARTHRITIS-INTERSTITIAL LUNG DISEASE

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Telomeres are specific regions of repetitive nucleotide sequences that protect chromosome ends and preserve genetic information. During each cell division, telomeres shorten, leading lastly to cell cycle arrest and apoptosis. The telomere-associated protein (telomeric repeat-binding factor 1 [TERF1]) is essential for their maintenance. Shortened telomeres have been described in Rheumatoid Arthritis (RA) and in idiopathic pulmonary fibrosis. Furthermore, few data are present in the literature regarding telomere length (TL) in RA-associated interstitial lung disease (ILD), while no data are available regarding TERF1 in RA. Therefore, our aim was to evaluate the TL and TERF1 gene expression levels in RA patients with and without lung involvement.

Eighty-nine RA patients with (RA-ILD) and without (RA-noILD) lung involvement and twenty-one age- and sex-matched healthy controls were enrolled. Genomic DNA and total RNA were isolated from peripheral blood mononuclear cells. Relative TL was measured using qPCR assay, which quantifies a ratio of telomeric repeat copy signal and a reference single-copy gene signal. Expression analysis of TERF1 was performed by qPCR assay. ANOVA was used to compare mean TL and TERF1 expression data among the different phenotypic groups. A multivariate logistic regression analysis was used to correct the P-value for sex, age and disease duration.

TL was significantly shorter in all RA patients compared to controls (P= 0.0016). In particular, RA-ILD patients showed significantly shorter TL compared to both controls (P= 0.00001) and RA-noILD (P= 0.0006). The association between TL and RA-ILD was confirmed after multiple corrections for sex, age and disease duration (P 0.001). In RA-ILD, TL correlated negatively with disease duration (P= 0.007, R= -0.408). TERF1 expression levels were reduced in RA compared with controls (P= 2.17E-17), also after stratification in RA-ILD patients and RA-noILD patients (P= 3.37E-10 and P= 2.78E-10, respectively). In addition, in female patients TERF1 expression levels correlated positively with TL (P= 0.004, R= 0.328).

These results show that the alteration of TL and TERF1 expression levels in AR patients is more evident in presence of ILD. Our hypothesis is that oxidative stress, known to characterize the pathogenesis of pulmonary diseases, could accelerate telomere shortening in these patients.

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GENETICS OF COMPLEX DISEASES

MASSIVELY PARALLEL REPORTER ASSAY (MPRA) COMBINED WITH BAYESIAN FINE MAPPING APPROACHES PRIORITIZE VARIANTS AND THEIR POSSIBLE FUNCTIONAL MECHANISM ASSOCIATED WITH MULTIPLE SCLEROSIS (MS) FROM GENOME WIDE ASSOCIATION STUDIES (GWAS)

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Multiple Sclerosis (MS) is an autoimmune multifactorial disease affecting central nervous system.

Large-scale Genome Wide Association Studies (GWAS) detected 200 MS risk loci. Linkage disequilibrium (LD) represents a limitation of GWAS in pinpointing the likely causative variant among the large number of associated SNPs, thus fine-mapping studies are required. Functionally informed fine-mapping was performed using Paintor and CaviarBF on 36 known MS regions showing a significant replication in an Italian cohort with genotypes imputed against HRC panel (5903 individuals, 4259 MS, 1644 HC), and overlapping with at least one drug target gene (Drug-Gene Interaction database v4.2 (DGIdb). We used GWAVA, CADD and FINSURF scores for variant annotation and Open Targets Genetics tool for variant-to-gene mapping. For 5 regions, we functionally analysed all the SNPs in LD (r20.75) with the lead SNP using Massively Parallel Reporter Assay (MPRA), a high-throughput in vitro screening method able to test thousands of sequences for their putative transcription regulation role. Mpralm tool applied with an in house script gave us a value (LogFC) of the difference in expression. Analysis validation was tested performing one experiment in replicates. Further analysis done with MotifbreakR allowed the selection of TF that bind in the presence of a specific allele. Additionally, we confirm this selective binding by using tools such as ENCODE, RegulomeDB.For 11 regions, Paintor and CaviarBF prioritized at least 1 SNP with evidence of causality (posterior inclusion probability, PIP 0.75), and 5 of these SNPs target a drug target gene. For 4 regions, MPRA analyses identified at least one SNP influencing gene expression of a drug target gene, with a statistically significant effect. 2 of these regions replicate perfect results for 2 probes and similar results for other 10 probes, changing only in the p value. Rs72924108 emerges above all with selective binding in the presence of the C allele of the ZNF754 TF a paralog of the YY1, which is involved in the regulation of transcription of RNA-polymerase II.Although these findings need validation through in vitro methods, they potentially affect our understanding of disease mechanisms and their drug targets.

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RARE DISEASES

SAY–BARBER–BIESECKER–YOUNG–SIMPSON SYNDROME DUE TO DE NOVO PATHOGENIC VARIANT ON KAT6B GENE

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INTRODUCTION: Say–Barber–Biesecker–Young–Simpson syndrome (SBBYS) (MIM #605880) is a hereditary disease, autosomal dominant mainly characterized by blepharophimosis, ptosis, bulbous nasal tip, nonexpressive face, prominent cheeks, long thumbs, long great toes, heart defects, dental and thyroid anomalies, and delayed development. We report a pediatric case with a nonsense genetic variant in the K (lysine) acetyltransferase 6B (KAT6B) gene.

CASE PRESENTATION: Female at 9 months old, is the second child of nonconsanguineous parents. She was born at full term with a birth weight of 3210 g. Pregnancy was normal and delivery was complicated by meconium-stained amniotic fluid. She shows upslanting palpebral fissures, telecanthus, epicanthal fold and protruding tongue were noted at birth therefore suspected possibly Down syndrome. Normal results were obtained from neonatal echocardiogram and array CGH.

She was evaluated by medical geneticist identified flat facial profile, sparse eyebrows, bilateral blepharophimosis, upslanting palpebral fissures, telecanthus, epicanthal fold, bulbous nasal tip, prominent cheeks, prominent tongue, perioral hypotonia affecting the drooling and perioral erythema, normal female genitalia, proximally implanted thumb, long fingers and fifth finger clinodactyly in hands.

Whole exome sequencing (WES) was performed identified de novo heterozygous KAT6B c.5786CG (p.Ser1929*).

DISCUSSION: The evaluation of the child with major anomalies and/or minor multiple anomalies requires systematic examination that includes all the body systems for analyzed diagnostic possibilities through recognizable patterns and support with laboratory techniques as molecular cytogenetics or sequencing.

It`s important to consider that in patients with congenital abnormalities don`t give a hasty diagnosis based only on some patient data on initial impression, because an erroneous diagnosis can be given that labels the patient with a genetic disease that they do not actually have.

In this case, achieving an accurate diagnosis of SBBYS syndrome involved analyzing the correlation between specific anomalies and pathogenic variants on KAT6B gene: craniofacial and hands phenotype in absence of knee and genitalia defects to discard another diagnosis.

The high phenotypic variability of the KAT6B gene suggests a wide spectrum of genotype–phenotype correlations. Nonetheless, distal mutations of this gene have been associated with the genotype of SBBYS syndrome.

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CANCER GENOMICS

EXPLORING MENDELIAN-INFORMED GENETIC VARIANTS FOR THEIR POTENTIAL TO ALTER CANCER RISK USING THE UK BIOBANK

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Height is a well-established risk factor for cancer development: each 10cm increase in height correlates to a ~10% increase in cancer risk. There does not appear to be a simple explanation for this association, reflecting the genetic complexities of both height and cancer. Mendelian (single gene) syndromes have tremendous power to potentially better understand such complex associations. The relationships between Mendelian overgrowth syndromes and cancer have been studied extensively, with the majority conferring increased cancer risk. Conversely, we know far less about growth-restricting syndromes and cancer risk, however, one pituitary dwarfism, Laron syndrome, was recently identified as having a protective effect against cancer development. This observation prompted our hypothesis that other growth-restricting syndromes are also protective against cancer.

Our research utilised the expansive phenotypic and genetic data in the UK Biobank to explore potential associations between variants in genes associated with growth-restricting syndromes, and cancer risk. First, we optimised in silico predictive algorithms to identify likely deleterious variants in short stature and microcephaly genes. Using pathogenic variants reported in publications or ClinVar, and benign variants based on multiple homozygous occurrences in gnomAD, we found that BayesDel consistently outperformed other algorithms, including CADD, REVEL, and AlphaMissense. The use of variant pathogenicity scoring algorithms informed the construction of UK Biobank cohorts consisting of participants with short stature and microcephaly-associated variants. These cohorts were investigated for associations with cancer-related data fields, including incidence, type, and age at diagnosis using logistic regression analysis. Through this research, we aim to understand more broadly the protective role variants in these disease-linked genes might play, enabling more precise risk prediction in an era of personalised medicine.

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COMPUTATIONAL BIOLOGY AND AI

BEYOND MISSENSE: EXPANDING APOGEE`S REACH TO TRNA VARIANTS IN MITOCHONDRIAL DISEASES

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Mitochondrial DNA (mtDNA) variants are implicated in numerous human diseases. However, their heteroplasmic nature, varying mutational loads across tissues, and diverse clinical presentations, with onsets spanning any life stage and affecting multiple organs, complicate the assessment of their pathogenicity. To address this challenge, the 2020 ACMG-AMP joint consensus recommendations recognized APOGEE as the sole AI-based variant effect predictor (VEP) offering supporting evidence for missense variant pathogenicity (PP3) or benignity (BP4). APOGEE 2, the most recent iteration, solidified its superior classification performance against modern missense VEPs, including deep learning models such as AlphaMissense. Notably, it exhibited a moderate correlation with the established mitochondrial local constraint (MLC) score, highlighting its unique insights.

In contrast, mtDNA variants encompassing tRNA genes do not have a definitive VEP, despite accounting for 46% of all disease-associated variants listed in MITOMAP. Existing tools are either outdated or archived, creating a critical gap in our ability to assess their potential disease contribution. We addressed this need by expanding APOGEE`s capabilities to encompass tRNA variants. Our novel tRNA VEP utilizes a Balanced Random Forest classifier trained on a manually curated dataset incorporating evolutionary, positional, structural, energetic, and sequence-based features. This classifier takes into account the imbalanced nature of our training data, which contains more benign than pathogenic variants.

To ensure optimal performance, the learning procedure included an out-of-bag prediction based on hyperparameter optimization (tuning). The training-tuning procedure was tested using cross-validation repeated 3 times with different dataset partitions. The resulting AI-powered method has an auROC of 0.8 and an auPRC of 0.5, far outperforming all other VEPs in these and other metrics. We then used the trained model to score all possible tRNA single nucleotide variants and used Bayesian reasoning to determine pathogenicity probabilities. This entailed calculating a precise heteroplasmy-dependent prior pathogenicity probability using supervised Bayesian optimization on population databases.

The pathogenicity scores and probabilities of missense and tRNA variants are easily accessible via MitImpact (https://mitimpact.css-mendel.it), a collection of genomic, clinical, and functional annotations for all nucleotide changes in human mitochondrial genes, providing researchers and clinicians with a valuable tool for comprehensive mtDNA variant analysis.

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RARE DISEASES

Carrier screening for Spinal Muscular Atrophy 1 in women who are in the first trimester of pregnancy

Carrier screening for Spinal Muscular Atrophy 1 in Brunilde Persia[1], Arianna Allegretti[1], Francesco La Rocca[1], Catia Fausto[1], Carmela Centoducati[1], Giovanni Stella[1], Francesca Simone[1], **Carrier screening for Spinal Muscular Atrophy 1 in Domenico Dell'Edera**[1]
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Background

Spinal muscle atrophy (SMA) is a relatively common autosomal recessive neuromuscular disease that leads to progressive muscle degeneration until exitus. The frequency of SMA carriers varies by ethnicity, so the American College of Medical Genetics (ACMG) recommends population screening for SMA carrier status. In this work, we carried out the screening of SMA carriers in the Basilicata region. Molecular screening for the SMN1 gene was offered, in conjunction with prenatal tests for the screening of major chromosomal diseases, to 1.160 pregnant women between April 2021 and January 2024.

Methods

The number of copies of the 7 and 8 exons of the SMN1 gene was detected using quantitative PCR in real-time (qRT-PCR), and the results were confirmed by multiple amplification with a ligation-dependent probe (MLPA).

Result

We found 32 women carriers of SMA (frequency 1:36). For this reason we extended the examination to their respective husbands who were all found not to be carriers of SMA. These couples were informed that the fetus has a 50% risk of being carrier of SMA. Furthermore, we have extended the molecular examination in the blood relatives of the carriers by activating genetic surveillance. We communicated to the remaining 1128 women found not to be carriers of SMA that in any subsequent pregnancies they will not have to carry out the test with a significant saving in socio-economic terms.

Conclusion

Our workflow turns out to have significant advantages:

- It is performed in the first trimester of pregnancy in conjunction with tests to screen for the main chromosomopathies.

- Women who are found not to be carriers of SMA1, in the case of subsequent pregnancies, will not be tested with considerable economic savings.

- If the woman is a carrier of SMA1, the examination is extended to her husband. If the latter is a carrier of SMA1, the couple is offered the possibility of studying the SMN1 gene in the fetus.

If the fetus is affected by SMA1, it will be able to benefit, as soon as it is born, from adequate pharmacological therapy that corrects the genetic defect.

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RARE DISEASES

HLH IN CARD11-GOF WITHOUT LYMPHOCYTOSIS: EXPANDING THE SPECTRUM OF BENTA DISEASE

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The CARD11 gene encodes a scaffold protein involved in NF-kB signaling, which controls peripheral B-cell differentiation and several critical T-cell effector functions. Its mutation causes the BENTA disease (B cell Expansion with NF-kB and T cell anergy), an autosomal dominant disorder characterized by onset in infancy of splenomegaly and polyclonal expansion of B cells, resulting in peripheral lymphocytosis.

Less than 30 patients with CARD11 Gain-Of-Function (GOF) mutations have been published, with the main clinical features being lymphoproliferation and splenomegaly, recurrent respiratory tract infections, and susceptibility to viral infections. Autoantibodies are frequently detected and autoimmune cytopenias are common as far as marked B lymphocytosis in childhood, even if several cases have been reported without this clinical sign.

We describe a novel heterozygous GOF mutation of CARD11 in a family with three affected members. The patients showed most of the clinical features previously described in BENTA, but none of the three had significant absolute B lymphocytosis.

The index case has been followed up for recurrent infections, hypogammaglobulinemia was found and immunoglobulin replacement therapy was started. The mother of the proband at the age of 44 developed a Diffuse Large B cell Lymphoma. The daughter of the proband at the age of five underwent adenotonsillectomy and blood tests highlighted progressive increase of IgM. A lobar pneumonia progressively led to multiple organ failure. Bone marrow aspiration demonstrated hemophagocytosis, HLH was diagnosed and the patient underwent bone marrow transplant.

Our three patients confirm that, although frequent, B lymphocytosis is not necessarily present in all the patients, hence the acronym BENTA may be misleading due to a more complex and heterogeneous immunological phenotype. In light of these findings, we suggest CARD11 GOF as a less confusing and more appropriate name to label this disease.

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GENETICS OF COMPLEX DISEASES

Gene regulation of immune response in monocytes in atherosclerosis

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Motivation and Aim: The impairment of human monocytes and macrophages in responding to inflammatory stimulation is a notable aspect of atherosclerosis. In normal circumstances, inflammation should promptly resolve through mechanisms like tolerance developed from repeated stimulation, resulting in lower cytokine secretion during subsequent stimuli. When compromised, this may lead to chronic inflammation. Previous research suggested mitochondrial mutations linked to atherosclerosis contributing to immune response impairment in cybrids (THP-1 cells with patient mitochondria). This study employs a transcriptome analysis to investigate signaling pathways in normal (tolerant) and intolerant cytokine secretion responses.

Materials and Methods: Cybrids, with varying mtDNA mutation burdens, underwent 1000 ng/ml LPS stimulation for 4 hours, followed by PBS washing and medium refreshment. A second 20-hour LPS stimulation was applied, and the resulting supernatant was analyzed for TNF, IL-1b, and CCL2 using ELISA. RNA from cybrids, pre- and post-LPS stimulation, was sequenced using Illumina NovaSeq 6000. Transcriptome analysis, using DESeq2 and geneXplain platform, identified DEGs and master regulators with specific parameters (Score 0.2, FDR 0.05, Z-score 1.0) based on assembling interaction networks using GeneWays database.

Results: In evaluating the pro-inflammatory response of cybrids, the secretion of four cytokines (CCL2, IL8, IL6, and IL1b) was measured. These specific cytokines were selected due to their distinct roles in the inflammatory response and differing mechanisms in responding to inflammatory stimuli. DESeq2+geneXplain analysis identified DEGs for all 4 cytokines, unveiling dozens of master regulators consistent across them. For normal response three master down-regulators (DLG3, GRIN1, MMP2) were identified, while intolerant response revealed six up-regulators (ZBTB32, OSM, LBR, IL3, IL16, FANCA). No common master regulator post-second stimulation was observed.

Conclusion: Six up-regulated master genes (ZBTB32, OSM, LBR, IL3, IL16, FANCA) and three down-regulated master genes (DLG3, GRIN1, MMP2) were identified in intolerant and tolerant immune responses, respectively. Subsequent knockdown experiments aim to explore their roles and assess the immune response in genetically modified cells. These in-depth studies hold the potential to unveil crucial diagnostic markers and identify novel pharmacological targets, ultimately contributing to the effective prevention of the chronicization of inflammation.

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PRECISION HEALTH

1+MILLIONGENOMES' EU INITIATIVE AND THE ITALIAN PLAN TOWARD PREVENTIVE AND PRECISION HEALTH

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The '1+MillionGenomes' (1+MG) EU initiative aims to enable secure, cross border access to genetic profiles and associated clinical data across EU Member states, to foster the application of genomics in clinical practice and empower healthcare and research. Italy was among the signatories states of the 1+MG declaration; is actively engaged in 2 strategic EU-funded projects: 1) "Beyond 1 Million Genomes" (B1MG), to provide a conceptual blueprint of methodology and quality standards for genome sequencing, infrastructural and technical architecture for sharing data, ELSI policies and metadata standards; 2) "Genomic Data Infrastructure" (GDI), for the deployment of a federated ecosystem for genome and health-data, compliant with B1MG provisions and is among proponents of the "Genome of Europe" (GoE) project, aiming to build a public repository of at least 500,000 genetic profiles of healthy Europeans.

The European effort aims to build an infrastructure with appropriate governance rules to allow secure access to genomics and corresponding clinical data across Europe for fostering research, personalized healthcare and better health policy development/planning.

The Italian plan is coordinated by Italian Ministry of University and Research (MoUR) and Italian Ministry of Health (MoH) with the collaboration of the Italian node of ELIXIR, The European Research Infrastructure for Life Sciences, and with the established 1+MG/B1MG National Mirror Working Groups, supported by a grant by the Centre for Control of Diseases (CCM) of MoH.

Another relevant national project, to be integrated in the European ecosystem for human data, is "Health Big Data" (HBD) financed by MoH with 55M euros over 10 years, aiming at implementing a national infrastructure for the sharing of clinical and omics data between Italian Research Hospitals (IRCCS) and help setting up regional centers for health electronic records. HBD is coordinated by Alleanza Contro il Cancro (ACC), in collaboration with Politecnico of Milan. Other stakeholders are BBMRI-ERIC, the European Research Infrastructure for biobanking, the Italian Institute of Technology (IIT), the National Research Council (CNR) and the Human Technopole (HT) in Milan.

We will present the state of art of ongoing activities for the sharing of clinical and omics data in Italy.

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GENETICS OF COMPLEX DISEASES

INTEGRATIVE ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES AND EPIGENOME DATA FOR IDENTIFICATION OF NOVEL GENETIC FACTORS FOR CRANIOFACIAL MORPHOGENESIS

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Genetics is a major contributor to our craniofacial morphology. Craniofacial malformation is a congenital birth defect with heterogeneous genetic contributions. There are currently many genome-wide association studies (GWAS) for craniofacial malformation. However, due to its complex genetic contribution and clinical presentations, the genetic contribution and diagnostic criteria remain elusive.

We collected data from publicly available GWAS datasets from craniofacial malformation and normal control (GSE69664, GSE248483, GSE141901, and GSE74100) from the Asian population. Most of the patients demonstrated unilateral anomaly (90%) with an anomaly in external auditory meatus. For multiome data, we collected publicly available histone marks (H3K27ac, H3K4me1, H3K4me2, H3K4me3, H3K27me3, and H3K36me3) chromatin immunoprecipitation sequencing (ChIP-seq) data from developing human embryo facial prominence across four to six post-conception weeks. We scored each single-nucleotide polymorphism region (SNPs) by adjusting the GWAS P-value weighted by additional information layers from normalized ChIP-seq peak height. We used the mean area under receiver operating characteristic (AUROC) curve to evaluate the performance of each method with 50 iterations and 70:30 training-validation splits.

To evaluate the optimal machine learning methods for disease SNPs prioritization, we compared various methods, including logistic regression, random forest, XGBoost, and support vector machine. We found logistic regression method performed the best among these methods, and the baseline AUROC is 0.88 without an additional information layer. We accessed different combinations of epigenomic marks and developmental stages. We found the information layer from H3K4me2 and H3K4me3 ChIP-seq signals from post-conception week 4 performed optimally by improving the value of AUROC to 0.936. Retrospective inspection of our trained model revealed rs16951723, rs7543281, rs2701520 were novel SNPs associated with craniofacial malformation.

In this study, we discovered novel candidate gene loci that contribute to craniofacial morphogenesis. Analysis of these gene loci, and transcriptional regulation of these genes in human populations will allow us to understand the genetic architecture of the normal range facial variation.

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TRANSGENERATIONAL INHERITANCE / EPIGENETICS

Evaluating the efficiency of low DNA inputs for methylation profiling using the Illumina Infinium MethylationEPIC BeadChip

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Background: DNA methylation (DNAm) is among the essential epigenetic mechanisms controlling gene expression. DNAm also regulates transposable elements, X-chromosome inactivation, and genomic imprinting. Methods for DNAm detection include the Illumina Infinium MethylationEPIC BeadChip. However, there is currently limited published evidence to establish the minimum amount of DNA that can be employed while maintaining data quality. In fact, low DNA inputs affect data reliability. The current study evaluated the impact of low DNA inputs on array performance across various individuals.

Methods: Peripheral whole blood samples were obtained from six healthy donors. DNA was extracted using QIASymphony DSP Midi kit and QC'ed with Nanodrop and Qubit fluorometer. Four DNA input amounts were used, specifically: 250 ng, 40 ng, 10 ng, and 5 ng. Bisulfite conversion was performed with the EZ DNA Methylation Kit. Bisulfite-converted samples were run on the Illumina Infinium workflow following the manufacturer's instructions. Samples were run in duplicates.

Results: The test sample indicates that even when the DNA input is significantly reduced to 50 times less than Illumina's recommended 250 ng input, the assay continues to effectively detect a substantial number of CpG loci. However, when the DNA input falls below 10 ng, the data becomes less reliable, exhibiting low reproducibility and increased variability. We have also assessed both intra- and inter-individual variability among the samples.

We observed a decrease in data reliability and an increase in noise as the DNA input dropped to 10 ng, leading to noticeable declines in data quality. Across the six individuals, the detected CpG count with 40 ng of DNA input showed lower variability and higher reproducibility. Conversely, both 10 ng and 5 ng DNA input amounts resulted in a higher variability.

Our study supports the use of lower amounts of DNA for an efficient methylation assessment through the Illumina Infinium MethylationEPIC BeadChip; this is particularly relevant when there is a need to use precious or limited samples.

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RARE DISEASES

DNA LIGASE IV DEFICIENCY: AN UNEXPECTED GENETIC CHARACTERIZATION FOLLOWING A TRIPLE NEGATIVE BREAST CANCER DIAGNOSIS

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Human cells have developed different DNA-repair pathways to handle the challenges posed by DNA damage. Whenever these pathways are damaged, the entire genomic stability is compromised. DNA double-strand brakes are particularly dangerous, as if not correctly resolved they can drive mutagenesis events leading to carcinogenesis and apoptotic mechanisms. Here we report an unexpected diagnosis concerning compromised repair of double-strand breaks due to a malfunction in the non-homologous end-joining (NHEJ) pathway.

The patient sought consultation at our Breast Unit following a diagnosis of breast cancer and a history of medullary aplasia with severe adverse drug reactions induced by pre-transplant conditioning chemotherapy. The cancer was originally classified as a sarcomatous. Following surgical excision, a thorough immunohistochemical assessments was conducted. The tumor displayed negativity for progesterone and estrogen receptors and for the human epidermal growth factor-2. A monophasic metaplastic triple-negative classification was eventually assigned. Additional analysis included: (i)fluorescence in-situ hybridization (FISH) (ii)fibroblasts karyotype (iii)cytotoxicity assays to evaluate the response to different chemotherapies. FISH analysis was conducted due to the initial classification of the tumor as “sarcoma”, however no fusion genes typical of Edwig Sarcoma were detected. Karyotype analysis revealed 16% of metaphases with abnormal karyotypes, showing aneuploidies and unbalanced structural rearrangements. Cytotoxic assays performed following the onset of acute toxicity in response to chemotherapy, revealed high sensitivity to cisplatin and oxaliplatin, but resistance to paclitaxel.

Considering the patient`s young age (35 years) and the tumor classification, a clinical exome sequencing was requested.

The initial focus on genes linked to hereditary breast and ovarian cancer yielded no causative variants. A second virtual-panel targeting medullary insufficiency-associated genes revealed a pathogenic variant in the LIG4 gene (NM_206937.2:[c.833GA];[c.833GA]) which encodes DNA ligase IV, an enzyme crucial for the NHEJ pathway and V(D)J mechanism of somatic recombination that occurs in developing lymphocytes during the early stages of T and B cell maturation.

The homozygous LIG4 variant explained the observed medullary aplasia, the heightened sensitivity to radiation, the hypo-pigmented spots, and the early onset of breast cancer strongly supporting the conclusive diagnosis of DNA ligase IV deficiency, an exceptionally rare disorder with an estimated prevalence of 1/1.000.000.

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GENETICS OF COMPLEX DISEASES

EXPLOITING THE POTENTIAL OF BASE EDITORS TO ASSESS THE BIOLOGICAL RELEVANCE OF SAMD9-MUTATED MDS: A PEDIATRIC CASE REPORT

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A 3-years-old boy from Guatemala referred to our hospital due to severe hepatitis and trilinear cytopenia. Microbiological tests excluded infective diseases, while the clinical and laboratory findings did not fulfill the diagnostic criteria of Hemophagocytic Lymphohistiocytosis. After performing a bone marrow (BM) histological biopsy, the diagnosis of Refractory Cytopenia of Childhood (RCC) was made. RCC is a peculiar type of pediatric Myelodysplastic Syndrome (pMDS), characterized by compromised hematopoiesis and BM hypocellularity, with an elevated risk of acute myeloid leukemia progression. The patient underwent clinical exome sequencing specific for pMDS, uncovering a germline mutation in Sterile α motif domain-containing protein 9 (SAMD9) gene (c.4496AG), classified as Variant of Uncertain Significance (VUS). Germline mutations in SAMD9 and SAMD9-Like (SAMD9L), two paralogue genes located on chromosome 7, encompass the most common pMDS-predisposing genetic alterations. SAMD9 and SAMD9L are pivotal proteins involved in maintaining the homeostasis of hematopoiesis regulated by IFN-α. SAMD9/SAMD9L gain-of-function (GOF) mutations seem to induce an excessive anti-proliferative and pro-apoptotic effects in hematopoietic stem cells under the IFN-α stimulus. To establish the pathogenetic role of our patient's SAMD9 mutation (c.4496AG), we recreated it in vitro by exploiting a GA base-editing approach in K562 cell lines. This cell line exhibits triploidy of chromosome 7, thus enabling the possibility to evaluate the graduate cumulative effects of 1, 2 or 3 edited SAMD9 alleles. The mutation was obtained in a bulky population of K562 cells, with negligible bystander effects. Then, cell clones carrying 1/3 or 2/3 edited alleles were selected and expanded in culture. Cells with 3/3 mutated alleles were unable to grow, thereby unveiling a strong anti-proliferative effect induced by the mutated SAMD9 protein. The RT-PCR expression SAMD9 levels under IFN-alpha stimulus was progressively higher in clones carrying 1/3 and 2/3 mutated alleles, as compared to unmutated K562 cells, thus revealing SAMD9 overexpression as a potential mutation-induced GOF effect. The use of base-editors might offer an innovative in vitro platform for validating the pathogenetic impact of SAMD9/SAMD9L variants. Moreover, in future, a base-editing reverting strategy might be explored as a possible gene-editing therapeutic approach for tackling pMDS.

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RARE DISEASES

INTRA-FAMILIAL VARIABILITY IN INHERITED NFIA-RELATED DISORDER: LESSONS FROM TWO FAMILIES

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NFIA (MIM *600727) haploinsufficiency is associated with a rare condition variably characterized by neurodevelopmental disorders, central nervous system malformations, and genitourinary anomalies, referred to as NFIA-related disorder or “Brain malformations with or without urinary tract defects” (MIM #613735). It is usually due to de novo variants identified in probands with unaffected parents. We describe two families (A and B) with multiple affected individuals displaying markedly variable expressivity.

In family A, the proband was a male fetus presenting mild asymmetric isolated cerebral ventriculomegaly at 20 gestational weeks (gw). The 30-year-old father reported a personal history congenital hydrocephalus (surgical treatment) and congenital inguinal hernia. He had no diagnosis of neurodevelopmental disorders. Paternal MRI displayed partial agenesis of corpus callosum (pACC) and enlarged ventricles. After amniocentesis, fetal karyotype, Chromosomal Microarray Analysis (CMA) and exome sequencing (ES) on the trio and on the paternal grandparents were performed. Karyotype and CMA were normal. ES identified the pathogenic NFIA (NM_001134673) c.82_83dup, p.(Trp30HisfsTer28) variant in heterozygosity in both affected individuals, occurring de novo in the father. Fetal MRI at 27gw revealed a worsening of the picture, with massive bilateral ventriculomegaly, cortical atrophy, ACC and gyration anomalies. The couple opted for termination of the pregnancy.

In family B, the proband was a 4-year-old male child with moderate global developmental delay, dysmorphisms and macrocephaly and no genitourinary anomalies. MRI showed pACC. Both his parents had mild learning disabilities and normal brain and abdominal imaging. CMA did not identify pathogenic or likely pathogenic variants. ES identified the pathogenic c.343CT; p.(Arg115Ter) variant in NFIA in heterozygosity, inherited from his mother. The couple later had an affected female child, bearing the variant and displaying ACC. The variant was also identified in the maternal grandmother, with intellectual disability and behavioral anomalies.

The analysis of two pedigrees in which NFIA pathogenic variants are inherited, as opposed to the more common de novo cases, shows that the associated condition presents marked intrafamilial variability. For NFIA¸ genotype and familial history are not sufficient to define a prognosis, and care and follow-up must be individualized.

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RARE DISEASES

HIGH PREVALENCE OF LARGE, KARYOTYPE-DETECTABLE, CHROMOSOMAL ANOMALIES IN NEWBORNS PRESENTING WITH DYSMORPHISMS WITHOUT MALFORMATIONS, BORN FROM UNEVENTFUL LOW-RISK PREGNANCIES

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In recent times, great effort has been invested in the implementation of rapid genomic testing in critically-ill newborns, due to the possible therapeutic implications. However, the definition of risks for non-critical newborns requiring clinical genetics assessment are, at present, not defined.

Our aim was to define the risk for chromosomal anomalies in newborns assessed for isolated facial dysmorphisms, with normal prenatal ultrasound scans (US) and no malformation identified after birth, born after uneventful low-risk pregnancies, in which no invasive diagnostic tests were performed. This is a 2-year, prospective cohort from a structure with about 3500 births per year. Standard Karyotype analysis and Chromosomal Microarray Analysis (CMA) were proposed in all eligible cases after clinical evaluation.

Thirty neonates were enrolled. In 8 out of the 19 (42.1%) neonates a large chromosomal anomaly was detected by standard karyotype analysis and confirmed by CMA: 1 with Wolf-Hirschhorn Syndrome (46,XY,del(4)(p16.3p16.1), 1 with 18q isochromosome (46,XX,idic(18q)), 1 with a 46.6 Mb duplication (46,XX,dup(5)(q21q32)), 1 with chromosome 18 ring (46,XY,r(18)(p11.3q22.3)), 4 with trisomy of chromosome 21. In all cases diagnosed with trisomy 21, Non-Invasive Prenatal Screening (NIPS) was not performed during the pregnancy. In all these cases, facial dysmorphisms were highly specific. In all other newborns, NIPS for trisomies 13, 18, 21 and sex chromosome anomalies had been performed, with low-risk results and the maternal (or egg donor) age was under 35 years old. When excluding trisomy 21 cases, 4/15 (26.7%) newborns with isolated dysmorphisms had a large chromosome imbalance. CMA did not identify further, non-karyotype-detectable, imbalances.

The present cohort demonstrates that even isolated dysmorphisms in neonates can be associated with rare and severe chromosomal imbalances, despite the absence of structural anomalies and the low a-priori risk. Given the high risks, but low urgency due to the absence of critical illness and therapeutic approaches, standard karyotype analysis can still be considered a solid first-tier test in newborns with isolated dysmorphisms.

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CANCER GENOMICS

SINGLE NUCLEOTIDE POLYMORPHISMS AND CERVICAL CANCER SUSCEPTIBILITY IN GEORGIAN WOMEN

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Background: Cervical cancer (CC) is the third most common malignancy in women worldwide. Genome-wide-association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) that are associated with an increased risk of cervical cancer. In this study, we aimed to investigate the association between 13 SNPs and overall cervical cancer risk in Georgian population.

Methods: 50 patients diagnosed with cervical cancer and 50 healthy women had enrolled in this study. All participants signed informed consent forms, which were approved by the Ethics Committee of the Tbilisi State Medical University. We genotyped 13 SNPs: rs10103314; rs231775; rs7726159; rs635634; rs2304204; rs2853672; rs6897196; rs11263763; rs148261157; rs7579014; rs687289; rs749292; rs727479 using the TaqMaq assay (Thermo Fisher, USA).

Results: Our analysis, which utilized the R statistical software package showed that there was no significant association between ten SNPs and the susceptibility to CC. However, we found that rs7579014 (BCL11A G/A), rs7726159 (TERT C/A) and rs687289 (ABO T/A) were significantly related to cervical cancer in Georgian patients ($p=0.02$, $p=0.04$ and $p=0.01$, respectively).

Conclusions: This initial study was the first to analyze Single Nucleotide Polymorphisms (SNPs) in women with CC in Georgia. SNP analysis may serve as a crucial marker for early susceptibility and detection of various cancers including cervical cancer. In our study three SNPs were demonstrated to be potential biomarkers for identifying cases of high risk for cervical cancer. However, further study with a higher sample size is required to confirm these findings.

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CANCER GENOMICS

ESTABLISHMENT OF AN IN VITRO SYSTEM FOR THE CHARACTERIZATION OF BRAFV600E SPLICING VARIANTS: REF AND X1.

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BRAFV600E is an oncogenic kinase involved in the MAPK pathway and, as a strong melanoma driver, it is targeted in melanoma therapy. Despite the combination of BRAFi together with MEKi, patients often develop resistance, enlightening the necessity of alternative strategies for melanoma treatment (PMID:32540454).

Starting from the assumption that a more effective targeting of BRAF cannot be achieved without a more profound knowledge of the regulation of its expression, in our lab we discovered a new BRAF splicing variant. BRAF-X1 is expressed in cells together with the isoform considered the reference one (BRAF-ref) and, even if it's not the most studied one, it's indeed the most abundant and most conserved (PMID:25685929, PMID:28454577, PMID:37393328).

At the mRNA level, the two splicing variants harbor 3'UTRs that differ in sequence and length (ref: ~100-4000nt vs X1: ~1300-7000nt). We also identified miRNAs and RBP that are positive and negative regulators of X1 expression (PMID:30929607, PMID:37013641).

Both isoforms are translated into proteins with different C-terminal sequences (ref: GYGAFPVH vs X1: -GYGEFAAFK, PMID:28454577, PMID:30237439), and our zebrafish melanoma models indicate that such sequences can provide isoform-specific functions with an impact on oncogenic potential (PMID:37393328).

The fine characterization of the two splicing variants is challenging as they are co-expressed. To overcome this problem, we are setting up an in vitro system in which, in a controlled fashion, we can induce the expression of one isoform only in the cancer cell line of interest.

In details, we aim to use an RNAi approach, based on an inducible shRNA, to down-regulate both endogenous BRAF isoforms, in combination with the CRISPR/Cas9 system to achieve a stable, inducible expression of the BRAFV600E isoform of interest (previously made insensitive to RNAi through genetic code degeneration).

Our preliminary tests conducted in HeLa and A375 cancer cell lines confirmed that the two techniques work correctly independently, so we are now proceeding with their combination.

Once obtained our doxy-inducible customized in vitro model, we aim to perform cell profiling and Co-IP studies to identify differentially regulated pathways as well as isoform-specific interactors that could unravel new drugs to improve BRAFV600E targeting, hence melanoma patients' treatment.

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PRECISION HEALTH

IN-VITRO GUIDED PERSONALIZED THERAPY OF CYSTIC FIBROSIS: CFTR FUNCTION RESCUE IN CF PATIENT-DERIVED ALI-CULTURES AND ORGANOIDS

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Cystic Fibrosis (CF) is caused by defects of the CFTR gene. CFTR-modulating drugs may overcome specific defects, as the case of Kaftrio, that proved strong ability to rescue the most frequent F508del variant function, even in genotypes with the mutated allele in single copy. Nevertheless, most rare genotypes lacking F508del allele are still not eligible for targeted therapies. We setup the nasal conditionally reprogrammed cells (CRC)-derived in vitro models of CF for the characterization of CFTR variants and the evaluation of drug response to obtain hints for future personalized CF therapy. We generated and validated cells derived from different patients carrying different genotypes, with the aim to assess theratyping. The effectiveness of treatments was evaluated by several functional tests. The CFTR response to Kaftrio was confirmed in F508del homozygous cells. Non-responding genotypes include mutated alleles with stop codons, as the W1282X, and the N1303K pathogenic variant. Drug responsiveness was obtained in the rare L1077P and I444T pathogenic variants. Basal CFTR protein levels were very low while mature CFTR increased following Kaftrio correctors exposure in L1077P-bearing genotypes. Forskolin-induced organoid swelling and Ussing Chamber assays congruently proved L1077P variant function rescue by Kaftrio. Notably, this rescue takes place even in the context of single-copy L1077P allele. Corresponding assays in W1282X/W1282X genotype demonstrated that this variant cannot be rescued by Kaftrio, as a consequence of heavily compromised CFTR protein expression. The effect seems to be due to the mRNA degradation, as demonstrated by rescue after combined treatment with Kaftrio and SMG1 inhibitor. Moreover, genotypes carrying the N1303K pathogenic variant responded to Kaftrio with different extent, based on the patient-specific CFTR protein/mRNA expression. In line with what observed for F508del-bearing genotypes, our findings could open the way for Kaftrio therapy for L1077P homozygous patients. Then, the possibility of single-allele treatment arises also for rare genotypes, with an allele-specific modulation as part of the mechanism. Interindividual variability in the CFTR expression should be considered in the planning of modulatory therapies. The theratyping of CFTR pathogenic genotypes may allow the clinical translation of effective therapeutic strategies.

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TRANSGENERATIONAL INHERITANCE / EPIGENETICS

AN EPIGENETIC AMPLIFICATORY THERAPEUTIC APPROACH FOR CYSTIC FIBROSIS: CFTR AND FOXI1 GENE EXPRESSION ENHANCEMENT BY DNA HYPOMETHYLATION

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Cystic Fibrosis (CF) is an autosomal recessive disease caused by mutations in the CFTR gene. CF has a high complexity at cellular level, as demonstrated by the recent amazing finding of the FOXI1-expressing ionocyte, which has been added to the already complex cellular context of lung. Innovative patient-specific cellular models are useful for approaches of epigenetic manipulation and drug testing (theratyping). We setup a patient-specific cellular system of nasal epithelial stem cells (called CF-CRC-AESC), inducible to respiratory differentiation, to test gene expression and epigenetic modulation. The targetable amount of CFTR may be variable and it may be usefully enhanced by amplificatory and ionocyte-inducing experimental therapies. CFTR and FOXI1 mRNA expression in brushing samples and CF-CRC-AESC cultures from CF patients, carriers and wild-type individuals revealed a great interindividual variability, mostly unrelated to the CFTR mutated genotype as well as to the FOXI1 SNPs profile. This variability can affect the therapeutic response. Moreover, the expression pattern of CFTR in patients is correctly reproduced in differentiated CF-CRC-AESC, highlighting a possible epigenetic control of CFTR and FOXI1 gene transcription, as well as of respiratory epithelium differentiation. Focusing on the therapeutic aspect, we tested an epigenetic amplificatory strategy by treatment with the hypomethylating drug 3-deazaadenosine (3-DZA) aimed to the enhancement of the mRNA expression of CFTR (CF-causing marker) and FOXI1 (marker of respiratory epithelium differentiation) genes in CF-CRC-AESC. The 3-DZA amplificatory therapy showed able to enhance both the CFTR and FOXI1 expression, in differentiated CF-CRC-AESC with wild-type and F508del/F508del genotypes. The mRNA levels of CFTR gene, and possibly also of FOXI1 gene, seem crucial variables for the enhancement of modulatory therapy. The 3-DZA hypomethylating agent could be exploited as an amplifying drug, since it showed to be able to amplify the gene expression of both CFTR and FOXI1, with possible increase of protein production. The increased amount of CFTR protein, even if hypofunctional due to pathogenic variants, could provide an increased substrate for CFTR correctors and/or potentiators. These findings provide new insights into the role of DNA methylation in the genotype-phenotype relationship in CF, as well as into epigenetics as a new CF therapeutic strategy.

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TRANSGENERATIONAL INHERITANCE / EPIGENETICS

EPIGENETICS MEDIATES HYPERHOMOCYSTEINEMIA IN REGULATING MOLECULAR PATHWAYS ASSOCIATED WITH ALZHEIMER'S DISEASE

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Late Onset Alzheimer's Disease (LOAD) is a multifactorial disease and although some polymorphisms have been linked to LOAD, they contribute only minimally to the overall risk. Under this perspective, exploring epigenetic regulation of molecular pathways associated with neurodegeneration can be an interesting approach. Elevated levels of homocysteine (hyperhomocysteinemia – HHcy) are a risk factor for AD. As individuals age, increased homocysteine levels often result from deficiencies in B vitamins, crucial co-factors in "one-carbon metabolism". This metabolic pathway sustains transmethylation reactions, including DNA methylation. Alterations in this pathway, either due to B vitamin deficiency or direct supplementation with the methyl-donor S-adenosylmethionine (SAM), have been shown to alter the Methylation Potential (MP), leading to changes in DNA methylation at specific genes. Our past research demonstrated that modulation of "one-carbon metabolism" and homocysteine levels regulate the expression and promoter methylation of several genes involved in amyloid production. Since amyloid accumulation in the brain results from an imbalance between production and clearance, we decided to investigate the impact of "one-carbon metabolism" on genes associated with amyloid transport and neuroinflammation. Specifically, we examined the response of LRP1, PICALM, IL-1β, and IL-6 to hypo-methylating conditions (B vitamin deficiency) or hyper-methylating conditions (SAM supplementation) in SK-N-BE human neuroblastoma and U87 human glioblastoma cells. Our findings revealed that alterations in one-carbon metabolism epigenetically modulate these genes, as well as the miRNAs involved in their regulation, in glioblastoma cells. Analysis of the methylation status of the promoters of these genes in glioblastoma cells, as well as the expression and methylation study in SK-N-BE, are underway. These findings offer insights into the methylation-dependent regulation of amyloid clearance and neuroinflammation, highlighting the multiple effects of changes in "one-carbon metabolism" on neurodegeneration.

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RARE DISEASES

EXOME SEQUENCING CAN BE IMPLEMENTED SUCCESSFULLY IN A LOW RESOURCE SETTING – FINDINGS FROM THE DECIPHERING DEVELOPMENTAL DISORDERS IN AFRICA (DDD-AFRICA) STUDY

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Developmental disorders represent a spectrum of often severe disabilities that are present from birth or early childhood. Due to the clinical and genetic heterogeneity of developmental disorders, diagnosis is challenging. This is exacerbated in Africa, where a lack of services and infrastructure for genomics compounds the problem. Exome sequencing (ES) is a proven approach to increase clinical utility and diagnostic yield for developmental disorders, but the feasibility of implementing this in a resource constrained setting has not been fully explored. The Deciphering Developmental Disorders in Africa (DDD-Africa) study aims to address this knowledge gap. ES was performed on 500 African patients and their parents (where available) at the Wellcome Sanger Institute. Samples were sequenced using Illumina NovaSeq 6000 paired-end sequencing, to a mean depth of 55x. Clinical and variant data was integrated using DECIPHER and interpreted by a multidisciplinary team of scientists and medical geneticists. Presented here are results from a first analysis focused on known developmental disorder genes and copy number variants. A mean of 91,468 variants were identified per individual, of which ~915 were rare and predicted to be putatively damaging, notably higher than what was previously reported in European cohorts. Of the identified variants, 1,050 were not present in current databases. We identified the first African patients for a range of known genetic syndromes. We illustrate how the addition of African data expands the phenotypic spectrum for developmental disorders and resulted in a diagnostic yield of 37%. Our findings demonstrate the discovery potential of ES for developmental disorders in a resource constrained environment, offering families the end to their diagnostic odyssey and future directed management.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

FUNCTIONAL POLYMORPHISMS OF SEROTONIN TRANSPORTER AND THEIR IMPACT ON ALCOHOL ADDICTION

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A large body of studies have underlined the important role of genetic variants not only in metabolic pathways, but also in the neurobiology of alcohol dependence, mediated by the neuronal circuits regulating reward and craving. Serotonin transporter (5-HTT), encoded by SLC6A4 gene, is targeted by antidepressant drugs and plays a pivotal role in serotoninergic transmission, and it has been linked to psychiatric diseases and alcohol dependence. Transcriptional regulation and expression of 5-HTT, depends also on sequence variations occurring in exons, intron/exon regions and in untranslated regions in 5' and 3'; being the second, important for the splicing machinery and the last for the binding of transcription factors and micro RNAs. Between the more studied, there are the serotonin transporter-linked polymorphic region 5HTTLPR (Long or Short allele, respectively 14 or 16 repeats) and the single nucleotide polymorphism (SNP) rs25531 (A or G allele) that act together as a tri-allelic polymorphism (La-Lg-S alleles; Lg and S with low function). This work intends to shed light on the role of sequence variations of 5-HTT in alcohol dependent subjects compared to control individuals. To do this, we evaluated the allelic and genotypic frequencies of 17 SNPs (using PCR and a mini-sequencing assay), already known in literature to affect the expression and/or the function of 5-HTT, comparing a population of 1447 alcohol dependent (AD) subjects to a control group of 441 abstemious individuals. Moreover, we performed a haplotype analysis, with the aim to evaluate possible differences between populations concerning these sequence variations. We found a difference, statistically significant, in allelic ($p=0.0083$) and genotypic ($p=0.0151$) frequencies concerning the tri-allelic polymorphism, with the higher function alleles and genotypes more represented in the control population. Furthermore, selecting haplotypes with a frequency above 5% in at least one of the categories in study, we found that three (ATGCCCCCCTCCACA1612; ATTCCCCCCTCCACA1610; GTGCCCCCCTCCACA1412) were more frequent in control population (p0.0001) and one (GTGCCCCTCTCCACA1412), more frequent in AD subjects (p0.0001). While the tri-allelic polymorphism has been extensively examined in alcohol dependence, even if with contrasting results, the role of haplotypes requests further studies to be clarified.

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RARE DISEASES

Rare disease coordination in the french part of Switzerland

Alessandra strom[1], Layla Motchane[2], Dre Loredana D'Amato Sizonenko[3], Dre Christel Tran[1], Prof. Marc Abramowicz[2]

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Abstract: This poster will present the rare disease platform that has been developed to facilitate the access to undiagnosed and/or specialized consultations in the French speaking part of Switzerland, as listed in the Orphanet knowledge database. This platform represents a partnership between Geneva and Lausanne University Hospitals. The poster will also present the rare disease center activities and its collaboration with the national coordination (kosek) in order to implement specialized rare disease reference centers and improve interdisciplinary care pathways for patients.

Useful links: https://www.kosekschweiz.ch/fr/kosek

https://orphanet.site/switzerland

https://www.chuv.ch/fr/medecine-genetique/gen-home/patients-et-familles/nos-consultations/centre-maladies-rares

https://www.hug.ch/medecine-genetique/maladies-rares

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RARE DISEASES

DE NOVO HOMOZYGOUS In2G VARIANT IN CYP21A SHARED BY TWO SIBLINGS WITH CONGENITAL ADRENAL HYPERPLASIA

Chong-Teik Lim[1], Tzer-Hwu Ting[2], King-Hwa Ling[1,3], Yee-Lin Lee[2], Nurul Huda Musa[1], Noor Haliza Mohamed Ibrahim[1], **Assoc. Prof. Dr. Karuppiah Thilakavathy**[1,3]
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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder in which enzymes in the cortisol biosynthesis pathways are disrupted. The most common cause of CAH is 21-hydroxylase deficiency (21-OHD) due to variants in the CYP21A gene. It is characterized by decreased cortisol and aldosterone synthesis and excessive androgen production. This study aims to identify the genetic variants responsible for CAH in a family with two affected children. The proband, a 3-year-old boy, presented with salt-losing crisis at day 6 of life with biochemical and hormonal profiles confirming CAH. The condition also affects his 5-month-old sister who was born with ambiguous genitalia, while both parents and two older brothers are unaffected. Whole-exome sequencing (WES) was conducted on the proband`s DNA, and the identified variant was validated through Sanger sequencing. WES genetic analysis revealed a homozygous pathogenic variant, NM_000500.9:c.293-13CG (In2G), representing the most common CYP21A2 changes related to the severe form of 21-OHD due to the altered reading frame of the gene producing a non-functional enzyme. Subsequent genetic screening of the entire family disclosed the father as a heterozygous carrier, while the mother and brothers exhibiting a homozygous wild type. The affected sister has the same genotype as the proband and presents the same phenotype. Given that one defective allele was inherited from the father, the presence of the other allele is likely due to maternal germline mosaicism. These findings not only contribute to the molecular understanding of CAH but also emphasize the importance of comprehensive genetic analyses, particularly in unraveling de novo or inherited variants, in an effort to offer better genetic counselling.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

VARIANTS OF UNCERTAIN SIGNIFICANCE IN NEURODEGENERATIVE DISEASES: A MASSIVE APPROACH TO DISSECT THEIR EFFECT ON SPLICING MECHANISM

Beatrice Piola[1], Giovanni papagni[1], Fjorilda Caushi[1], Nicola Pomella[1], Faezeh Sojoudi Ghamnak[1], Francesco Favero[2], Davide Corà[3], Luca Magistrelli[4], Alfredo Brusco[5], Fabiola De Marchi[6], Letizia Mazzini[6], Sandra D'Alfonso[7], Lucia Corrado[7]

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Background/Objectives: Neurodegenerative disorders have a genetic etiology either in the familial and the sporadic forms. Next Generation Sequencing (NGS) identified many new genetic causes, although many cases remain unsolved. This missing heritability can be partially explained by variants classified as „Variants„of Uncertain Significance" (VUS), as these variants could alter splicing mechanisms. The high-throughput Massively Parallel Splicing Assay (MaPSy) was developed to test the impact on splicing mechanism of hundreds of variants in the same assay. With this study, we aim to analyze by a modified-MaPSy a subset of VUS identified by Whole Genome Sequencing (WGS) in 140 patients affected by different neurodegenerative diseases.

Methods: From the variants identified by WGS in a panel of 855 neurodegenerative disease genes, we used to prioritized SpliceAI score 0,6 and a Minor Allele Frequency (MAF) 0,001. An amplicon-based library, covering exonic, intronic-flanking, and splicing regions, was cloned into the pSPL3 plasmid and transfected into HEK293T cells. NGS analysis was performed, paired-end reads were aligned to a reference genome and BAM files were manually inspected.

Results: Starting from all the variants found in a large panel of 855 genes, 53 were prioritized based on SpliceAI score (0,6) and having a MAF 0.001. Moreover, we selected those variants whose prediction was confirmed by a second tool (NNsplice) and did not overlap with restriction sites. At the end, we performed MaPSy on 19 variants. Among them, 6 variants located in canonical splicing sites, 7 exonic variants (4 synonymous and 3 missense) and 6 localized in intron sequences. Aberrant splicing isoforms were detected for 15 /19 variants. The aberrant splicing events were predicted to generate an out-of-frame transcript for 12 variants, an in-frame transcript for 3 variants, and unpredictability for one variant, due to its 5'UTR localization. Analysis for the remaining four variants was impaired due to low NGS locus coverage.

Conclusion: Our results showed that variant impact on splicing mechanism can be analysed by a massive approach and SpliceAI is an effective predictor for prioritizing splicing-altering variants. Further analysis must be performed to comprehend the role of splicing variants in disease mechanisms.

Grants: NEUrodevelopmental-Disorder-Genetics (NEUDIG)- PRIN20.

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PRECISION HEALTH

THE EFFECTS OF SULT1A1 COPY NUMBER AND THE AFRICAN-SPECIFIC CYP2D6*17 ON TAMOXIFEN RESPONSE IN SOUTH AFRICAN BREAST CANCER PATIENTS

Miss Bianca Kruger[1,2], Delva Shamley[3], Collet Dandara[1,2]
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Introduction: Tamoxifen, the established hormone therapy for oestrogen receptor-positive breast cancer, exhibits considerable effectiveness in reducing both cancer recurrence and mortality. Nevertheless, the response to tamoxifen treatment varies among patients, with recurrence observed in up to half of patients. This variability in response is partially attributable to genetic variation in genes that encode enzymes involved in tamoxifen metabolism. Specifically, genetic variation in CYP2D6 and SULT1A1, key players in tamoxifen metabolism, may significantly impact its efficacy. However, the pharmacogenetics of tamoxifen in populations of African descent are inadequately studied, presenting challenges in identifying the primary factors contributing to the observed variability in response. Notably, high (3) copy numbers of SULT1A1 have been reported to be more prevalent in African populations than in other populations. This study, therefore, aimed to investigate the impact of the African-specific variant, CYP2D6*17, and SULT1A1 copy number variation (CNV) on tamoxifen treatment outcomes in South African breast cancer patients.

Materials and Methods: A cohort comprising 170 Mixed and African Ancestry breast cancer patients from South Africa, all undergoing tamoxifen treatment, underwent genetic profiling for CYP2D6*17 using the Agena MassARRAY System. Associations between SULT1A1 CNV, CYP2D6*17 and five-year disease-free survival (DFS) was examined using log-rank tests and the Cox proportional hazards regression model.

Results: Carriers of the CYP2D6*17 allele exhibited a trend towards significantly lower five-year DFS (P=0.051) compared to individuals that do not carry this allele. Conversely, women with more than three copies of SULT1A1 had significantly higher 5-year DFS (P=0.010) than those with three or fewer copies.

Discussion: CYP2D6*17 and SULT1A1 CNV may have implications for tamoxifen treatment among South African breast cancer patients. This observation highlights the importance of incorporating additional variants specific to African populations and promoting a genome-wide approach to comprehensively elucidate the potential involvement of other genes in tamoxifen pharmacogenomics.

POSTER SESSION 2
APRIL 09
FROM 10:15 TO 11:00

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PRECISION HEALTH

SLC7A8 TRANSCRIPTIONAL REGULATORS IDENTIFICATION FOR THE TREATMENT OF AGE-RELATED HEARING LOSS (ARHL)

MS Paola Tesolin[1], Paola Bartoccioni[2,3,4], Giulia Pianigiani[1], Maria Caballero[2], Anna Morgan[1], Cristina Bon[1], Gema Rojas Granado[2], Israel Ramos[2], Patrick Aloy[2], Paolo Gasparini[1,5], Virginia Nunes[6,7], Giorgia Girotto[1,5], Manuel Palacin[2,3,4]

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Background: ARHL is a common multifactorial sensory impairment affecting 30% of individuals over 65 years old, but still, there is no targeted therapy approved. Indeed, few ARHL-causative genes have been identified. Our previous study (PMID:29355479) suggested SLC7A8 (NM_012244) as a novel candidate gene by proving that knock-out (KO) mice develop ARHL and by identifying damaging heterozygous missense variants in humans displaying ARHL. These results suggest that SLC7A8 is a promising target for developing a novel treatment.

Methods: We performed a high throughput screening (HTS) to detect SLC7A8 transcription regulators, considering that previous findings (PMID:29355479) suggested that high expression of functional SLC7A8 may be important for maintaining good hearing function. A reporter line expressing the luciferase under the transcriptional control of the SLC7A8 promoter was generated employing the CRISPR/Cas9 technology. In an entirely automated process, the reporter line was incubated for 48 hours with a library of 2240 approved drugs, 960 drug-like compounds (tested at a concentration of 1.5µM in duplicates), and 44 regulators of SLC7A8 transcription reported in the literature (tested in seven doses in duplicates).

Results: The HTS led to the identification of 56 hit compounds. The activity of the most promising hits was confirmed with a secondary independent HTS, which defined the dose-response curves with luciferase and cell viability assays. Furthermore, three compounds, which are already approved drugs, were further selected, employing the Chemical Checker algorithm (PMID: 32440005) and following literature research. In particular, Compound-1 mechanism of action has already been associated with increased SLC7A8 expression. Compound-2 was selected as a representative of a drug category highly present among the hits. Finally, Compound-3 is already used to treat other forms of hearing loss. An RT-qPCR was performed to test those molecules in different cell lines, confirming that the treatment with 1.5µM of the compounds increased SLC7A8 expression.

Conclusions: These results highlight the possible use of the most promising compounds in treating SLC7A8-dependent ARHL. Subsequent evaluations will include testing the hits in cell lines carrying the variants identified in the patients, in the inner ear-related UB-OC1 cell line, and in Slc7a8 heterozygous KO mice.

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RARE DISEASES

A novel inherited TBX3 missense variant in a prenatal case of ulnar-mammary syndrome shows wings defects in Drosophila melanogaster

Prof Irene Bottillo[1], Andrea D'Alessandro[2,3], Maria Pia Ciccone[1], Gianluca Cestra[2,4], Gianluca Di Giacomo[1], Evelina Silvestri[5], Marco Castori[6], Francesco Brancati[7], Andrea Lenzi[8], Alessandro Paiardini[9], Silvia Majore[1], Giovanni Cenci[2,3], Paola Grammatico[1]

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Ulnar mammary syndrome (UMS) results from heterozygous variants in the TBX3 gene and impacts limb, tooth, hair, apocrine gland, and genitalia. UMS is characterized by variable expressivity even within the same family, and no obvious genotype–phenotype correlations have been described to date. TBX3 belongs to the evolutionarily conserved Tbx gene family, characterized by the presence of a T-box DNA-binding domain.

Here, we describe a fetus presenting with severe upper limb reduction defects, in which we identified a novel heterozygous c.400CT (p.P134S) missense variant in TBX3. The mother, who remained clinically misdiagnosed until this pregnancy, was found to carry the same variant c.400CT alteration. To study the functional effect of the p.P134S alteration, we generated the first Drosophila humanized model for UMS, creating transgenic flies expressing TBX3-P134S, along with three additional TBX3 variants selected from the gene's mutational spectrum. Literature revision was conducted with the attempt to uncover the full-blown TBX3 clinical and mutational spectrum.

Phenotypic analysis of the Drosophila model, coupled with in silico modeling of the mutant TBX3 proteins, suggested that the p.P134S is a bona fide novel UMS-causing alteration impacting TBX3 localization. Comparative analyses of developmental defects caused by different TBX3 Drosophila mutants, indicated that missense changes in the T-box domain are more likely to be pathogenic as compared to those located outside this regulatory region. Additionally, most TBX3 pathogenic variants reviewed in the literature clustered in the T-box domain.

To aid clinicians with evaluation clues and ease UMS recognition, we estimated the frequency of the main clinical features of the disease. We outlined core features often present pre-pubertally, including defects of the ulna and/or of ulnar ray, hypoplastic nipples and/or areolas and, less frequently, genital anomalies in young males.

These results enhance our understanding of the molecular basis and the clinical spectrum of UMS shedding light on the functional consequences of distinct TBX3 variants in a developmental context.

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RARE DISEASES

Integrated approach for the molecular characterization of 486 Italian patients affected by different forms of hereditary cardiovascular diseases.

Professor Stefania Zampieri[1,2], Stefania Zampieri[1], Alessia Paldino[3], Beatrice Spedicati[1,2], Daniela Mazzà[1], Giovanni Turchetto[1], Matteo Dal Ferro[3], Gianfranco Sinagra[2,3], Stefania Lenarduzzi[1]

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Introduction

Hereditary cardiovascular diseases (hCVDs) represent a major cause of morbidity and mortality worldwide, encompassing a wide and heterogeneous spectrum of heart conditions, such as cardiomyopathies, channelopathies, arrhythmic and vascular disorders.

Aim of the study

This study aims to provide a detailed genetic picture of hCVDs in a deeply characterized cohort of Italian patients.

Materials & Methods

Whole Exome Sequencing (WES) was performed in 486 subjects affected by hCVDs, recruited at the Medical Cardiological Unit of the Cattinara Hospital (Trieste, Italy). Both single nucleotide and Copy Number Variants (CNVs) were identified by WES analysis. CNVs were further studied either by MLPA or other high-throughput technology (i.e.,MinION).

Results

Eighty-nine percent of patients were affected by cardiomyopathies, 7% by channelopathies, 4% by aortopathies, and pulmonary arterial hypertension.

WES allowed the identification of a molecular diagnosis in 23% of patients, with a significative higher detection rate in familial forms than in sporadic ones (41% vs 16%). 52 novel causative variants within 22 genes known to be involved in hCVDs were identified.

Besides TTN, which carried the largest number of variants (n=30), the most frequently mutated genes were MYH7, MYBPC3, FLNC, PKP2 and DSP, explaining 78% of all solved cases.

The recurrent variant (c.913_914del) within MYBPC3 gene was identified in two patients, affected by hypertrophic cardiomyopathy and coming from Veneto region (Italy), thus confirming a previously described founder effect. Moreover, we expanded the clinical spectrum of hCVDs, identifying variants either in minor genes for hCVDs or in patients with an unspecific cardiomyopathy, highlighting the genetic heterogeneity of the disease.

Moreover, we identified a deletion in PKP2 gene in three patients further validated by MLPA, and a novel large deletion, including DSG2, DSC2, and TTR genes, analysed by MinION technology which allowed us to characterize the deletion breakpoints.

Conclusion

This study underlines the importance of a multi-step genetic diagnostic approach (WES, MLPA, and MinION) to characterize hCVDs patients, thus emphasising the complexity of the genetic background of

hCVDs and providing crucial information for risk stratification, clinical management, prognosis, and recurrence risk estimation.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

A DEEP MUTATIONAL SCANNING FRAMEWORK TO DECIPHER THE MOLECULAR BASES OF BRCA1 DEFICIENCY IN BREAST CANCER

Sig Gennaro Gandolfo[1], Lorenzo Vaccaro[1,2], Francesco Panariello[1], Rosa De Santis[1], Mirella Massa[1,2], Antonio Grimaldi[1,2], Tonya Fusco[1,2], Davide Cacchiarelli[1,2,3]

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In recent years, one of the biggest challenges for clinical genetics is to correctly classify genetic variants; nowadays the gold standard to address this problem is represented by in silico predictors. Despite that, these tools often result in inaccurate predictions of variants, placing them in a larger group of Variants of Uncertain Significance (VUS). To handle this issue, we decided to apply Mutagenesis by Integrated TilEs (MITE), a novel, highly multiplexed approach to site-directed mutagenesis (SDM). MITE can lead us to encode all possible variants in a desired region of a gene, to decipher the effect of each potentially pathogenic missense variant, overtaking the limits of in silico predictors. We applied this Deep Mutational Scanning approach to the mutagenesis of BRCA1, an oncosuppressor gene linked to Breast and Ovarian Cancer predisposition and a major player in Triple Negative Breast Cancer arising, with 85% of its variants classified on Varsome as VUS. The BRCA1 protein plays a crucial role in repairing DNA double-strand breaks in conjunction with PARP family proteins. Thus, we hypothesized that inhibiting PARPs with Olaparib, an FDA-approved drug, would result in the accumulation of numerous DNA damages and subsequent cell death in cells lacking functional BRCA1. To validate the efficacy of this approach, we treated UWB1.289 cells (a BRCA1 null cell line), transduced with BRCA1 wt or empty vector, with Olaparib. After a few days of treatment, we observed the survival of UWB1.289 cells transduced with BRCA1 wt, while those transduced with the empty vector underwent cell death. The Neutral and LoF variants identified through this system will be tested by functional assays (e.g., CA, Comet, yH2A assay) and compared with clinical data from major cancer databases (e.g., OncoKB) to confirm the robustness of the method. This approach represents a milestone in cancer research, providing the clinical community with a robust resource to evaluate the effects of mutations in one of the most important oncogenes.

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RARE DISEASES

IDENTIFICATION AND FUNCTIONAL EVALUATION OF AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING IMPAIRMENT GENES IN RWANDA

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The incidence of hereditary hearing impairment (HI) is higher in developing countries compared to developed countries. Globally, more than 120 independent genes have been identified as responsible for almost 50% of profound HI. Nonsyndromic HI is the most common form accounting for 70% of cases of which 80% are autosomal recessive. Reported mutations in GJB2, GJB6, and GJA genes are the most common cause of HI globally but studies among Cameroonian and South African participants did not identify a significant association, hence the need for further genetic exploration of other responsible genes in the African population. In Rwanda, more than 50% of HI among children has been attributed to hereditary causes but no genetic evidence has been established yet. Our study aimed to use community-based nationwide recruitment to determine the genetic etiologies of hearing impairment in Rwanda. We recruited 26 cases of HI, and 100 control individuals without HI or a family history of HI. Participants with early onset HI were included after clinical examination, including audiological assessment by pure tone audiometry and/or auditory brainstem response to exclude exposure to ototoxic drugs or infections including prenatal exposure. Peripheral blood was collected from consented families (proband, both parents, affected sibling/or other relative and unaffected sibling). In simplex cases (only one affected individual in the entire family), blood samples were taken from the affected person, both parents and an unaffected sibling if available. Furthermore, samples were collected from 100 healthy hearing controls randomly selected from the Rwanda population. Families were whole exome sequenced (WES) and Sanger sequenced to call and validate variants. This study resulted in 17 known variants and 2 candidate novel variants responsible for HI in the cohort investigated. Myosin XVa (MYO15A), MYO7A, and TMC1 genes were the most predominant (34.6%, n= 9/26) associated with HI in our cohort. The next step will be to study the function of the candidate variants. We believe that this study will help to advance the science of HI and be the foundation to establish appropriate medical care for affected individuals and families in Rwanda.

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CANCER GENOMICS

CCND2 MUTATIONS IN ATYPICAL CHRONIC MYELOID LEUKEMIA: A POSSIBLE MARKER OF DISEASE

Dott. Giovanni Iaquinta[1], Michele Ragazzo[1], Silvia Angeloni[1], Maria Laura Bisegna[2], Serena Pasquali[1], Mara Rosaria Angelitti[1], Massimo Breccia[2], Paola Grammatico[1]

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Atypical chronic myeloid leukemia (aCML) is a rare MDS/MPN disease characterized by more than 10% of immature circulating precursors, as well as morphological dysgranulopoiesis, leukocytosis (13 x 109/L) and the absence of BCR::ABL1 rearrangement. Moreover, well known typical mutations associated with myeloproliferative disorders can be detected. Mutational landscape associated with this disease was recently described with frequent involvement of ASXL1, SETBP1, ETNK1 and EZH2 mutations. CCND2 mutations were rarely detected in MPN or MDS/MPN patients but have been found in AML, particularly in core binding factor leukemia. We performed mutational analysis using next-generation sequencing (75-genes panel by Archer VARIANTPlex™) of 7 prospectively recorded aCMLs. We identified specific somatic mutations as following: ASXL1 in all cases (100%), SRSF2 in 6 cases (87,7%) and SETBP1 in 5 cases (71.4%). Indeed, we also detected somatic alterations of CCND2 gene (encoding p.Thr280Ala, p.Pro281Ser and p.Pro281Thr alteration) in 3 cases (42.8%). The p.Thr280 and p.Pro281 are conserved amino acids involved in cyclin D2 degradation. The reported mutations are either missense or nonsense mutations which have been shown to increase the stability of cyclin D2 and hence the cellular proliferation. Clinical features of analyzed cases showed resistant leukocytosis to conventional treatment and aggressive clinical course. The biological consequences of CCDN2 mutations are still a matter of discussion: Lu Mei He and colleagues, demonstrated that the threonine 280-mutant cyclin D2 transgenic mice had greatly reduced β-cell apoptosis, with suppressed expression of pro-apoptotic genes. Indeed, Mou and colleagues, confirmed CCND2 overexpression results in the upregulation of the mTOR pathway, which may drive the progression of leukemia. In vivo murine models showed that Everolimus restored the non-leukemic cells and extended their survival time in CCND2wt wt and CCND2mut group, while an increased specific sensitivity to Palbociclib has been reported. In summary, these findings suggested a sign of genomic instability in Ph-negative atypical MPN with a possible strict connection to a faster progression to an AML-like phase. The identification of possible recurrent CCND2 mutations in patients with aCML would increase our understanding of the mutational landscape and would indicate a possible target for molecularly-driven therapies for this subtype of MDS/MPN.

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CANCER GENOMICS

GERMLINE SH2B3 MUTATION IN MYELOPROLIFERATIVE DISORDERS WITH ERYTHROCYTOSIS

Dott. Giovanni Iaquinta[1], Monica Rossi[2], Silvia Angeloni[1], Michele Ragazzo[1], Emanuele Savino[1], Elena Rossi[2], Patrizia Chiusolo[2], Emilia Scalzulli[3], Serena Pasquali[1], Paola Grammatico[1], Massimo Breccia[3]

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Idiopathic erythrocytosis (IE) comprises a heterogeneous group of disorders characterized by hyperplasia of the erythroid lineage. However, in many cases, the molecular basis remains undetermined. In clinical practice, the diagnostic evaluation of JAK2 unmutated erythrocytosis remains a challenge, and patients are frequently subjected to non-uniform testing. In patients with unexplained erythrocytosis, JAK2, MPL, and EPO receptor (EPOR) mutations are absent alongside subnormal serum Epo levels, while SH2B3/LNK exon 2 mutations or polymorphisms may be present. In one study, SH2B3 mutations were detected in 6 out of 112 (5.3%) patients with JAK2 negative IE. SH2B3 is a plasma membrane-bound protein which, among other functions, inhibits downstream signaling pathways of both wildtype and mutant JAK2 activity by binding to cytokine receptors and JAK2 through the SH2 domain. Here, we report a new heterozygous germline variant c.232GA (p.Glu78Lys) observed in 3 patients: a patient with Budd Chiari syndrome (BCS) and documented thrombocytosis (661000/mmc) and leukocytosis (10650/mmc), one with IE, and one with secondary myelofibrosis post essential thrombocythemia (SMF-ET) but with erythrocytosis. Next-generation targeted sequencing analysis (NGS) was performed and, respectively, the first patient showed SH2B3 germline variant (c.232GA, p.Glu78Lys - VAF 52%) and PROS1 (S protein) germline variant (p.Ser533Pro VAF 46%), the second patient showed only SH2B3 germline variant (c.232GA, p.Glu78Lys - VAF 51%) and the third patient showed MPL somatic variant (p.W515_P518delinsKT – VAF 35%) and SH2B3 germline variant (c.232GA, p.Glu78Lys - VAF 49%). In the patient with BCS, the hematocrit observed at diagnosis was 36%. This apparent normal value was probably masked by the thrombosis. In the second patient, the hematocrit at diagnosis was 54%. Both patients started antiaggregant treatment. In the third patient, the polyglobulia revealed an extreme thrombocytosis, probably related to MPL mutation. In conclusion, we identified a new heterozygous germline mutation in SH2B3/LNK gene that would increase our understanding of the molecular basis in IE and possible correlation with erythrocytosis manifestation.

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RARE DISEASES

ADVANCEMENTS IN GENOMICS: UNRAVELING INSIGHTS INTO RARE DISEASES FOR PRECISION HEALTH.

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In recent years, groundbreaking advancements in genomics have significantly transformed the understanding and management of rare diseases. This abstract explores how genomics is reshaping precision health globally, emphasizing its pivotal role in driving scientific progress and innovation. By leveraging comprehensive genomic analysis, researchers are gaining unprecedented insights into the genetic basis of rare diseases, facilitating personalized diagnostics, treatments, and therapeutic interventions.

With a focus on the HGM2024 theme, "Genomics in Precision Health," this abstract underscores the transformative potential of genomics education in advancing healthcare equity. Collaborative efforts across international consortia and research networks are accelerating the pace of discovery, offering new avenues for targeted interventions and improving patient outcomes.

Furthermore, the application of genomics in precision health has the potential to catalyze the diagnosis and management of rare diseases such as Alpha-1 Antitrypsin Deficiency (AATD). Through genomic analysis, intricate correlations between genetic variants and disease phenotypes can be elucidated, enabling early detection and personalized management strategies.

In summary, genomics holds promise in revolutionizing the future of precision medicine, unlocking new opportunities for diagnosis, treatment, and prevention in the realm of rare diseases. Its impact underscores the critical importance of integrating genomics into healthcare initiatives worldwide.

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GENETICS OF COMPLEX DISEASES

THE CASE OF A FEMALE PATIENT CARRYNG AN IKBKG MUTATION PRESENTING WITH IMMUNODEFICIENCY WITHOUT INCONTINENTIA PIGMENTI

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Background: Female carriers of IKBKG (encoding the NF-κB essential modulator - NEMO) gene mutations are generally asymptomatic but heterozygous amorphic mutations, resulting in the absence of NEMO function, could be responsible for incontinentia pigmenti (IP). Hypomorphic IKBKG mutations allowing a residual NF-κB activity sufficient for ectodermal development causes immune dysfunction in affected males. We report the case of a IKBKG female carrier without IP but with an unexpected infectious/immunodysregulatory phenotype.

Case Presentation: 37-year-old female carrier of a heterozygous IKBKG mutation (NG_009896:g.10503GT). This substitution revealed a G to T transversion at position +1 of the donor splice site in the 5′ untranslated region (UTR) of the IKBKG gene generating 2 abnormally sized NEMO mRNA species with intact coding sequences.

Her brother, affected by NEMO deficiency, died at the age of twenty years due to atypical mycobacteriosis and pneumocystis infection, whereas her two male maternal cousins, with the same mutation, are still alive, mainly suffering from immunodysregulatory manifestations. Both mother and maternal aunt are asymptomatic carriers. Our patient was healthy until the age of 25 when severe asthma, currently treated with omalizumab, and Hashimoto thyroiditis occurred. At the age of 35, HLAB27 positive ankylosing spondylitis was diagnosed and treated with infliximab for a few months. Chest-CT scan showed pulmonary cavitation in the upper left lobe. Mycobacterium Gordonae and galactomannan were detected by Broncho-Alveolar Lavage, requiring long antimicrobial therapy. Due to atypical mycobacteriosis and pulmonary aspergillosis an immunology consultation was requested.

Results: At immunological work-up B cell lymphopenia (5%), high serum IgA and IgE levels (392 mg/dl and 208 UI/ml, respectively) were found. WES, non-random X chromosome inactivation and expression of NF-kB signaling in fibroblasts are ongoing.

Conclusions: Our case suggests that the clinical phenotype of NEMO female carriers might be extended to infectious and immunodysregulatory manifestations with significant prognostic and counseling implications. Further genetic and functional studies will clarify whether the combination of other undisclosed pathogenic variants contribute to the phenotype spectrum and guide future steps in her management.

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CANCER GENOMICS

OVERVIEW OF THE GENETIC CAUSES OF HEREDITARY BREAST AND OVARIAN CANCER SYNDROME IN A BRAZILIAN PATIENT COHORT

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Identification of individuals with hereditary cancer syndrome have a great impact in cancer prevention, screening and treatment. Nevertheless, access to germline genetic testing in the Brazilian Universal Healthcare System is extremely limited. Therefore, detailed clinical information of patients referred for genetic tests in a real-life setting is scarce. We aim to characterize clinical, pathological and molecular features of a cohort of patients referred for germline testing in a public cancer genetics clinic. In this retrospective observational study, we retrieved clinical, pathological and molecular features of N=130 individuals that underwent germline testing by Next-Generation Sequencing of a commercial panel (25 genes). These individuals fulfilled testing criteria from the National Comprehensive Cancer Network Guideline for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic (Version 1.2022). Data collection was performed using a structure questionnaire in REDCap and data analyses was conducted in R. Out of 313 patients with breast, ovarian and/or pancreatic cancer initially screened to be tested, we selected 130 based on vital status, previous genetic testing, fulfilling NCCN criteria, time from first visit to genetics clinic. Out of 130 patients tested (128 females, mean age 49.1 years), 122 had breast cancer, 6 had ovarian cancer and 2 had pancreatic cancer. We identified 36 (26.7%) individuals with a germline pathogenic or likely pathogenic variant (PV) and 29 (22.3%) with a variant of uncertain significance. Five PV occurred in 1 patient (BRCA1:c.3331_3334del, N=4; BRCA1:c.5266dup, N=4; BRCA2: c.156_157insAlu, N=2; BRCA2:c.3046GT, N=2; and TP53:c.1010GA, N=3) and one patient had 2 PV (NF1:c.499_502del andATM:c.856CT). Factors associated with detection of PV were triple-negative breast cancer (OR 5.00 95%CI 2.06-12.14) and at least one first-degree relative with breast, ovarian or pancreatic cancer (2.56 95%CI 1.07-6.09). Interestingly, demographic features, age at cancer diagnosis and bilateral breast cancer were not associated with PV in this cohort. We identified 36 (26.7%) individuals with at least one PV in a cancer predisposing gene. Factors associated with hereditary cancer diagnosis were triple negative breast cancer and first-degree relative with breast, ovarian or pancreatic cancer but not age at diagnosis and bilaterality. These results contribute to understanding HBOC epidemiology in Brazilian public health system.

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RARE DISEASES

UNRAVELLING THE AGEING PHENOTYPE OF MDPL SYNDROME: CHARACTERIZATION OF PATIENTS' FIBROBLASTS

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Mandibular hypoplasia, Deafness, and Progeroid features with concomitant Lipodystrophy (MIM #615381) defines a very rare systemic disorder, named MDPL syndrome, due to a de novo in frame deletion in the POLD1 gene encoding the catalytic subunit of DNA polymerase δ.

In order to unravel the functional landscape, we established primary cell lines from MDPL human dermal fibroblasts (HDFs) obtained from patients of different ages (e.g. 8, 14, and 20 years). For each comparison among MDPL-HDFs and WT-HDFs, we used age-matched cells at increasing passage doublings, up to p20, to allow the age-phenotype correlation.

Besides having confirmed the reduction of POLD1 protein in mutated cells, we have already described several hallmarks of aging, such as genetic rearrangement, telomere shortening, cell senescence, proliferation defects and impairment of mitochondrial biogenesis and activity. In addition, considering Lamin A/C primary role in nuclear assembly, we examined the protein expression level, revealing a marked reduction in the older patients, as well as an impairment of Lamin B1, a known senescence marker.

In silico methods allowed us to identify possible interactors of POLD1 and bioinformatic approach reveals a binding interaction between POLD1 and the shelterin protein TRF1 (Telomeric repeat-binding factor 1), which is a negative regulator of telomere length. So, through an immunoprecipitation assay, we were able to confirm the interaction and importantly demonstrate an increased binding capability of mutated POLD1 variant to TRF1, respect to wild-type one. In addition, the expression of TRF1 resulted to be increased in older patients, predominantly at p20.

Since TRF1 is also a molecular interactor of PARP1 (Poly [ADP-ribose] polymerase 1), which is a surveillant of telomere length and involved in triggering DNA repair mechanism, we investigated PARP1 protein level. Thus, we revealed a significant decrease of protein level in all patients, particularly evident at p20, supporting the pleiotropic role of these proteins to aging phenotype.

Our experimental results have been crucial, as they allowed us to delve into the role of the POLD1 variant in MDPL, exacerbating premature aging phenotype through passages, and to provide a starting point for exploring pathogenetic mechanisms related also to other age-related conditions.

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GENETICS OF COMPLEX DISEASES

INTEGRATED OMICS PROFILE OF MONOZYGOTIC TWINS DISCORDANT FOR PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra. It is influenced by largely unidentified environmental factors and genetic components. Recent advancements in genetic research have provided insights into potential treatment and prevention strategies. However, the understanding of PD pathogenesis and the available therapies remain limited, emphasizing the need for early biomarkers and neuroprotective treatments. Traditionally, studies on monozygotic (MZ) twins have been instrumental in exploring the genetics of complex traits and diseases, thus making possible to control many potential confounders typical of general population studies (i.e., genetic background, early-life environmental exposure, age, and gender)

In this frame, we characterized 38 MZ twin pairs discordant for PD, with the aim to uncover signatures that could anticipate disease diagnosis. Utilizing various -omics approaches, including genomics, epigenomics, and transcriptomics, the final goal is to identify biomarkers and pathways associated with PD.

Considering that aging is the first risk factor for PD, we first assessed the effect of the disease on telomere length (TL), the main hallmark of cell senescence. Our results highlight that the disease duration impacts on the relative TL (RTL) ratio within twin pairs (≥10years 0.85±0.18 vs 1.06±0.22; P=0.015). Surprisingly, we also observed that PD-related mutations influence RTL (mean difference +0.30 [95%CI=0.14-0.46]; P=0.028), suggesting that aging drives idiopathic PD, emphasizing cellular senescence, while genetic forms are primarily initiated by disruptions of cellular pathways.

Then, we evaluated the somatic non-allelic homologous recombination (NAHR) landscape and we observed that patients with PD show a different signature of recombination when compared with their healthy twins; one of the main differences affect regulatory regions. Moreover, we observed that the disease duration has an impact also on the number of somatic NAHR events accumulated by the affected twins compared to the healthy counterpart (P=0.041).

These preliminary results and the planned integration of all the omics data collected for this cohort may lead to the discovery of biomarkers that could be used for early diagnosis or, as new therapeutic target/s, for personalized treatment of PD.

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RARE DISEASES

IMPROVEMENT OF ADPKD DIAGNOSIS AND PROGNOSIS: A MULTIDISCIPLINARY APPROACH

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In recent years, a multidisciplinary approach in medical research has significantly improved understanding of human genetic diseases, leading to advancements in clinical, molecular, genetic, and pharmacological realms. Next-Generation Sequencing (NGS) and bioinformatic tools have particularly propelled genomic medicine forward, aiding in diagnosis, prognosis, and personalized medicine. We employed a collaborative approach to improve Autosomal Dominant Polycystic Kidney Disease (ADPKD) management.

During the clinical process, 150 patients suspected of having polycystic kidney disease were recruited, and data from nephrology, genomics, and genetic susceptibility sections for each patient were collected in a specific structured database built using FileMaker software. It correlated family history, pharmacological therapies, physiological, radiological, hematological, and clinical data with genomic variants. Genomic DNA was sequenced utilizing the clinical exome panel on NGS platform (Illumina), yielding a diagnostic rate of 68% (102 patients). 71 patients were positive for PKD1 gene, 24 for PKD2, while 7 patients received a differential diagnosis with mutations in IFT140, ALG8, PKHD1, PRKCSH and UMOD genes. Approximately 19 % (29) revealed variants of uncertain significance (VUS) and the remaining 13% (19) did not present clinically significant variants. In negative patients' further investigation, using Multiplex Ligation-dependent Probe Amplification (MLPA) technique, revealed 3 patients positive to PKD1 large deletions, achieving a diagnostic yield of 70% (105 out of 150 patients). In silico evaluation of PKD1 missense variants, impacting the PKD repeat domains, have been conducted to delineate their effects on protein structure This involved comparing repeated PKD domains using computational methods. Moreover, ongoing in vitro functional assays are being conducted to investigate the consequences of PKD1-REJ domain missense variants on cleavage.

Collaborative efforts of different teams have led to successful protocols validating the benefits of a multidisciplinary approach, thus improving the ADPKD knowledge gap. The research project culminated in the establishment of Italy`s first-ever reference center dedicated to ADPKD patients. The integration of genomic data into clinical practice emerges as a crucial step, ensuring that patients can derive maximum benefits from personalized or precision medicine.

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RARE DISEASES

NEW METHOD TO IDENTIFY MYOTONIC DYSTROPHY TYPE 2 PATIENTS CARRYING VARIANT (TCTG)N REPEATS AT THE 3` ENDS OF THE CNBP EXPANDED ALLELES.

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Introduction: Myotonic dystrophy type 2 (DM2; MIM#602668) is an autosomal dominant multisystem disorder caused by (CCTG)n repeat expansions in intron 1 of the CNBP gene. Recently, our pilot study using Nanopore long-read sequencing, revealed the presence of a TCTG block at the 3′ end of the CCTG array not reported before in DM2 patients. Aim: To verify the presence of the (TCTG)n motifs in the CNBP expanded alleles in a large cohort of DM2-positive patients using an improved QP-PCR method for the selective amplification of TCTG-containing CNBP expanded alleles. Methods: We re-analysed 69 DM2 individuals previously characterized by a combination of PCR-based approaches (SR-PCR, LR-PCR and QP-PCR) to detect the presence of (CCTG)n expansions in the CNBP gene. To this purpose, we developed a modified quadruplet-repeat primed PCR (QP-PCR) method coupled to Sanger sequencing allowing the selective characterization of CNBP alleles containing TCTG blocks at the 3'-end. Results: Analysis of the TCTG motif by our modified QP-PCR revealed the presence of TCTG blocks at the 3′ end of the (CCTG)n array in 64/69 DM2 patients (93%), while only 5 DM2 patients (7%) showed the traditional motif of continuous CCTG expansions. Sanger sequencing of QP-PCR products generated using primer P4-TCTG further confirmed the presence of the (TCTG)n motif in all 64 DM2 patients. These preliminary data are now being validated by PacBio third-generation sequencing to fully characterize DM2 expansions in terms of (TCTG)n motif configuration and repeat composition. Conclusion: Taken together, this data could improve the genotype–phenotype correlations in DM2 genetic counselling and will be useful for patient stratification in future clinical trials.

Keywords: Myotonic dystrophy type 2, CNBP repeat expansions, QP-PCR, Variant Repeat

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

A FUNCTIONAL GENOMICS APPROACH TO DISSECT THE MOLECULAR BASES OF CDKL5 DEFICIENCY DISORDER

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As of today, the need for the discovery of the molecular bases of genetic disorders represents the main topic for both researchers and clinicians to identify novel, more efficient, and more specific treatments. Unfortunately, for rare and ultra-rare genetic syndromes, such as CDKL5 Deficiency Disorder (CDD), this process is still hampered. CDD is a dominant developmental disorder that leads to a large spectrum of neurological phenotypes. While the phenotype is almost clear, the lack of knowledge about the molecular mechanisms that lead to the disease and the limited cohort of diagnosed patients hampers the discovery of a real functional treatment. Today, in silico predictors often classify mutations as Variants of Unknown Significance (VUS), preventing an adequate treatment of patients and an efficient evaluation of phenotype. To address this point, MITE (Mutagenesis by Integrated TilEs) is a novel saturation mutagenesis technique that allows easy testing of thousands of potentially pathogenic protein variants in a single high-throughput biological assay coupled with Next Generation Sequencing (NGS). To this end, we propose to apply MITE to mutagenize the catalytic domain of CDKL5, a known hotspot for CDD. To interpret the activity of every possible disease-causative mutation of CDKL5, we hypothesized the use of CDKL5-/- SH-SY5Y as a platform for neuronal differentiation by providing Retinoic Acid (RA) and BDNF after the transduction with CDKL5 mutants (D0, D1, D5, D11). Samples were analyzed for RNAseq, and selected surface and intracellular antigens (IFI6, ITGB5, pEB2) underwent Flow-Cytometry analysis to determine the best candidate that would allow the sorting of cells transduced with active or inactive CDKL5 variants. Once validated, the selected antigen will allow performing a comprehensive analysis of the already generated CDKL5 variants and the consequent validation experiments to demonstrate the consistency of the molecular approach for mutation effect interpretation. In this context, a resource like MITE could be fundamental to allow for the prediction of the effect that CDKL5 variants could have on carrier individuals, allowing an early diagnosis and, consequently, an early treatment of symptoms. Moreover, the MITE platform for the CDKL5 catalytic domain could represent a strong platform for genetic testing.

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GENETICS OF COMPLEX DISEASES

Whole Genome Sequencing as a tool to identify genetic causes of adult-onset non-syndromic sensorineural hearing loss

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Background: Adult-onset sensorineural hearing loss manifests as bilateral progressive neurosensory loss of the auditory capacity, which is predominantly interested in medium-high frequencies. Although to date over 200 genes have been identified as contributing to human hearing loss, many more remain to be discovered.

The purpose of our study is to characterize variants, mainly rare (MAF 0.01%) and ultrarare (MAF 0,001%), at the level of genes associated, and not, with hearing loss responsible for the onset of the disease.

Methods: We enrolled 20 patients (5 males, age of onset 23-48 years, and 15 female, age of onset 15-53 years), with bilateral sensorineural hearing loss of no obvious etiology by medical history and audiological examination. Since genetic testing of GJB2 and GJB6 genes resulted negative, the DNA was subjected to whole genome sequencing (WGS). The data were processed through bioinformatic tools applying as filters the classification of variants (P/LP/VUS), MAF 0.01%, or unknown, and concordance of classification between the predictions in silico and the main databases considered.

Results: In the first level of analysis, we identified heterozygous variants of uncertain significance (VUS) in 4 probands at the level of the MYO7A, TJP2, DMXL2 and DIAPH1 genes, already associated with hearing loss. The family history was consistent with a dominant trait. An adequate segregation analysis could clarify the significance of the variants found. Furthermore, we identified a 45 years-old patient with previously undiagnosed DFNA3A.

Conclusions: The low frequency of predicted-pathogenic variants detected in known deafness-associated genes underlies the marked genetic heterogeneity of condition. For this reason, WGS represents a useful tool to investigate genetic cause in novel genes potentially related to the onset of presbycusis, unveiling new possible targets for the future development of innovative therapeutic and preventive approaches.

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RARE DISEASES

EXPLORING THE COMPLEX INTERPLAY BETWEEN NOTCH1 AND SERCA2 PROTEINS IN THE DARIER'S DISEASE: A PARADIGMATIC GENETIC NETWORK IN THE PATHOGENESIS OF A MENDELIAN GENODERMATOSIS

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Background/Objectives: Darier's Disease(DD) is a rare, dominantly inherited genodermatosis with the loss of desmosomal adhesion and abnormal keratinization, caused by variants in ATP2A2. ATP2A2 encodes for a SERCA2 protein, an ubiquitously expressed cellular pump responsible for calcium translocation from cytosol to endoplasmic reticulum. The molecular mechanism underneath ATP2A2 alterations is not known. Intriguingly, in other disease models SERCA2 is a master regulator of NOTCH1 signaling and SERCA2 inhibition causes NOTCH1 pathway inactivation. The project aims to define a DD transcriptional profile and the genetic, biochemical and physiological impact of DD ATP2A2 alterations on NOTCH1 signaling and/or on other new pathways.

Methods: we collected patients' skin biopsies: i)to perform transcriptomic RNA-sequencing analysis on affected and unaffected subjects; ii)to generate keratinocytes and fibroblasts primary cells for expression analysis and immunofluorescence assays of the NOTCH1-downstream effector targets (HES1, HEY1, c-MYC); iii)to investigate NOTCH1 signaling deregulations through immunohistochemistry on FFPE bioptic samples. Furthermore, we generated plasmids containing patients' specific ATP2A2 variants to evaluate their impact on the NOTCH1 signaling and the proteins interaction by performing in vitro functional and biochemical assays.

Results: We define a DD transcriptomic gene signature, focused on metabolic, ribosomal and immunological deregulations. We pointed out a molecular dependency of NOTCH1 pathways and ATP2A2 defects through the effects evaluation of patient-specific variant overexpression and by ex vivo assays based on patient-derived primary cell lines and tissue IHC.

Conclusions: By understanding the molecular network between SERCA2 and NOTCH1 in DD, novel and effective therapeutic approaches may be developed and tested in DD.

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GENETICS OF COMPLEX DISEASES

INCREASED BURDEN OF RARE VARIANTS IN GENES ASSOCIATED WITH MULTIPLE SCLEROSIS BY GENOME-WIDE ASSOCIATION STUDIES IN FAMILIAL MULTIPLE SCLEROSIS PATIENTS

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Multiple sclerosis is an immune-mediated neurodegenerative disease affecting the central nervous system with many known genetic risk factors. While genome-wide association studies (GWAS) have identified common MS-associated genetic variants, rare variants have also been shown to represent a significant portion of MS risk. However, the contribution of rare variants with larger effect sizes has not been systematically studied in MS yet. Moreover, we hypothesized that rare variants are more likely to contribute to familial MS (FMS). Therefore, we aimed to assess the burden of rare, predicted pathogenic (RPP) variants in genes associated with MS in GWAS in FMS and sporadic MS (SMS) patients compared to controls. Rare genetic variants in 111 GWAS-identified genes were assessed in 86 FMS, 89 SMS and 3868 control cases. The results show that RPP variants were significantly overrepresented in the FMS cohort whereas no significant enrichment was observed in the SMS cohort (p-values 5.27×10-74 and 1.00, respectively). Furthermore, within the FMS group, analysis revealed that six genes (ALPK2, ANKRD55, INTS8, IQCB1, JADE2, and MALT1) were significantly associated with an accumulation of RPP variants. In conclusion, we demonstrate that rare variants in genes identified by GWAS might contribute to genetic predisposition in familial MS patients.

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NON-CODING RNA GENES

FRG2A IS PART OF A NOVEL FAMILY OF LNCRNAS AFFECTING NUCLEOLAR FUNCTION IN FSHD CELLS BY A TRANS-ACTING EFFECT ON RDNA TRANSCRIPTION.

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Background/Objectives: Facioscapulohumeral muscular dystrophy (FSHD), a common hereditary myopathy has been associated with reduced copies of the D4Z4 macrosatellite at chromosome 4q35. Deletion of D4Z4 repeats leads to the inappropriate expression of 4q35 genes resulting in disease. Among 4q35 genes, FRG2A possesses unique features, being selectively overexpressed in FSHD patients and stabilized upon genotoxic noxa in inverted correlation with the number of D4Z4 repeats. We thus aimed at deciphering FRG2 gene function as a proxy indicator of 4q35 alterations.

Methods: Based on T2T genomic assembly we obtained a view of FRG2 paralogs interspersed within the human genome. By performing RNA fractionation/immunoprecipitation experiments we concluded that FRG2A belongs to a novel family of long non-coding RNAs associated with heterochromatin. To advance understanding of FRG2A transcript function, we performed an RNA-centric genomic profiling based on ChIRP technology.

Results:This strategy led to the identification of thousands of FRG2A target sites in the human genome and revealed FRGA2 association in trans to centromeres and to rDNA arrays. We also defined a large network of interactions affecting nucleolar function and architecture. Further analysis revealed that nucleolar function was altered in FSHD myoblasts with a significant impairment in rDNA transcription and in protein synthesis, which were both reverted upon FRG2 silencing.

Conclusion: Based upon these results we propose that deletion of D4Z4 leading to the inappropriate expression of the FRG2A lncRNA impacts ribosome function and protein synthesis in muscle cells. These observations open new perspectives on the molecular mechanism involved in FSHD pathogenesis.

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RARE DISEASES

RECOMBINANT INSERTIONAL TRANSLOCATION ASCERTAINED IN A 31-YEAR-OLD FEMALE WITH SYNDROMIC PHENOTYPE AND OVARIAN CANCER.

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Interchromosomal insertion translocations are considered rare rearrangements. Most of them are de novo and ascertained in subjects affected by neurological developmental disorders and congenital malformations. Few are inherited as recombinant rearrangement by an apparently balanced parent. We describe the case of a 31-year-old female presenting with dysmorphic features, developmental delay and mild learning difficulties together with other anatomical and functional disorders. She also received an early diagnosis of ovarian cancer. CGH-array analysis identified three chromosomal imbalances involving chromosomes 1 and 6 (a 3,10Mb deletion at 6p22.2, a 1,6Mb deletion at 1q43, and a 917kb duplication at 1q44). The proband's phenotype mostly overlapped that reported in cases with interstitial 6q21q22.1 deletion including the minimal overlapping region containing MARCKS, HDAC2, HS3ST5. Interestingly, SMYD3, one of the genes involved in the 1q44 duplication, have been found to be frequently overexpressed in ovarian cancer.

GTG-banded karyotype indicated an interchromosomal reciprocal insertion between chromosomes 1 and 6 which was confirmed by FISH. The (1:6) reciprocal insertional translocation was found in the healthy proband`s mother and brother who were found to carry the 1q43 deletion but had neither the 6q21q22.1 deletion nor the 1q44 duplication. Optical Genome Mapping (OGM) was performed to better characterize the chromosomal rearrangements in the family. The proband's OGM highlighted complex insertions characterized by multiple breakpoint junctions between chromosomes 1 and 6 with no additional loss at the insert sites. Furthermore, OGM provided detailed structural information on the de novo 1q44 duplicated fragment of 967 kb, which was inserted into 1q42 and joined to the proximal part of the inserted fragment of 3 Mb derived from chromosome 6 into the der(1). These findings are in line with the notion that complex rearrangements, such as interchromosomal reciprocal insertion, may result from catastrophic events of the chromoanagenesis type. It remains to be seen how frequently these events, some of which are certainly frequent in cancer cells, underlie constitutional genetic disorders. The question is not trivial considering the risk for a healthy carrier to have a child with syndromic phenotype due unbalanced recombination events of the complex rearrangement.

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NON-CODING RNA GENES

UNRAVELING THE ROLE OF LARIAT RNAs ACCUMULATION IN DISEASES ASSOCIATED WITH DBR1 LOSS OF FUNCTION

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RNA splicing is a crucial process in gene expression that entails the excision of introns from pre-mRNAs, leading to the formation of RNA intermediates containing lariat structures. The turnover of lariat introns relies on their rapid degradation, mediated by the RNA debranching enzyme (DBR1), which catalyzes the cleavage of the 2′–5′ phosphodiester linkage at the branchpoint. Notably, deficiencies in DBR1 have been implicated in various human pathologies marked by the accumulation of lariat RNAs, including cancer and brainstem viral infections, thus challenging the conventional view of introns as transient byproducts of RNA splicing. However, transcriptome-wide approaches and studies on lariat RNAs are currently limited. Herein, we introduce the development of a method termed DBR-seq, designed to isolate and sequence full-length spliced-out introns containing lariats. We aim at identifying lariat-containing RNA species potentially responsible for disease development that accumulate upon reduction of DBR1 loss-of-function. Since DBR1 knockout is lethal, we will take advantage of the auxin-inducible degron system to trigger acute DBR1 protein degradation in cellular models of epithelial and neural origin. To confirm the pathological relevance of our findings, we will also perform DBR-seq in neural cells derived from human H9 embryonic stem cell line harboring DBR1 I120T or Y17H point mutations found in patients.

Once we identify the main lariat-containing RNAs correlated with DBR1 loss-of-function, we aim to study the molecular mechanisms that link lariat RNA accumulation to disease onset by characterizing the subcellular localization of these RNAs as well as potential interacting molecules that may interplay with lariat RNAs in promoting disease progression.

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RARE DISEASES

PHENOTYPE-DRIVEN APPROACH:IMPROVING VARIANT PRIORITIZATION AND DIAGNOSIS IN A YOUNG MALE WITH AUTOSOMAL RECESSIVE SPINOCEREBELLAR ATAXIA

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We report on a young male of 19 years born from healthy non-consanguineous parents. He was an hospitalized patient presented with Leri-Weill syndrome, mild intellectual disability, Raynaud`s syndrome, polycythaemia, hypertension and hepatic steatosis. Neuroimaging revealed a globular appearance of the pituitary gland with a thickening of the pituitary stalk and a suspected hypersecretion of ACTH. He came to genetic and neurological examination at the age of 17th due to recurrent critical episodes characterized by generalized hypertonicity, fixed gaze, walking backwards, flexion of the trunk, nystagmoid ocular movements. At a later time, he also developed gait instability, postural tremor mainly in the right hand and visual disturbance. Given the complexity of patient`s phenotypic presentation, clinical exome sequencing was performed on the proband and his mother. Data were analysed using a phenotype-driven approach throughout Geneyx analysis software, prioritizing genetic variants based on the Human Phenotype Ontology (HPO). Parallel sequencing identified a potential compound heterozygosity (father missing) for two previously unreported likely pathogenic nonsense mutations (NM_016614.3 (TDP2): c.709CT (;) 357GA p.(Arg237Ter)(;)(Trp119Ter)) in TDP2 gene (OMIM: *605764). Pathogenic mutations in TDP2, encoding tyrosyl DNA phosphodiesterase 2, cause Spinocerebellar Ataxia autosomal recessive 23 (SCAR23, OMIM #616949), a very rare and progressive neurodegenerative disorder described in only nine patients to date, and caused by biallelic loss-of-function variants that result in greatly reduced or absent TDP2 protein. In conclusion, clinical presentation of our patient is very similar to the other reported ones except only for a later-onset ataxia (about 18 years old). Furthermore, our case broadens the mutational spectrum of the TDP2 gene, outlining also new genotype-phenotype correlations in a very rare condition. Moreover, it is also highlighted how a phenotype-driven clinical exome approach together with multidisciplinary collaboration between clinicians and laboratories, provides an effective tool to rapidly solve cases suspected of rare complex genetic diseases finally supporting appropriate medical management and genetic counselling.

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PRECISION HEALTH

POLYGENIC RISK SCORE IMPROVES THE RISK STRATIFICATION AND CLINICAL MANAGEMENT IN FAMILIES CARRYING PATHOGENETIC VARIANTS IN BREAST CANCER RELATED-GENES.

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Breast cancer (BC) is the most prevalent cancer among women and the identification of high-risk women together with early detection are crucial for improving good outcome rates. Positive family history of BC is considered an important risk factor for the disease prediction. Pathogenic variants (PV) in high- and moderate-risk genes, such as BRCA1/2 and PALB2 respectively explain about 20% of the familial risk. Moreover, a polygenic component including many variants of small effect contributes to the risk of developing BC in the general population and, notably, may also modify the individual risk in members of BC families. Thus, the combined effect of these variants, summarized as a polygenic risk score (PRS), can be relevant. With this aim, we performed a retrospective pilot study evaluating the PRS in women from BRCA1, BRCA2, and PALB2 BC families counselling at Policlinico Tor Vergata. We included in our study carriers and non-carriers of PVs and evaluated a PRS based on the analysis of 57.7113 associated variants (analyzed with my HealthScore by Veritas Intercontinental). We used CanRisk, an online software based on the BOADICEA v6 model, to calculate the adjusted lifetime BC risk. For carriers we considered 1) PV, 2) PV and PRS, 3) PV, PRS and family setting; for non-carriers PRS and family setting. Our data showed that in BRCA1/2 families the PVs have the major role in the stratification risk of BC and the PRS did not influence the final score. Conversely, PRS improves the BC risk estimate in non-carriers of these family. A different scenario may be observed in PALB2 families where PRS combined with PV gives a more informative lifetime risk for BC, such as age of cancer onset or risk of contralateral tumor for previously affected women. This study showed that in BC families, the PRS might help to concretely quantify the weight of the genetic familial background contributing not only to an individual risk stratification but also to a personalized clinical management for both PV carrier and non-carrier women in BC families.

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NON-CODING RNA GENES

FROM STRUCTURE TO FUNCTION: CHARACTERIZATION OF SINEUP RNA-ASSOCIATED RNP COMPLEXES DURING TRANSLATION

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Long non-coding RNAs (lncRNAs) are a widely expressed class of non-translated transcripts whose function and structure relationship are largely unexplored. They contain functional domains through which they regulate gene expression by interacting with proteins, DNA and RNA and forming ribonucleoprotein (RNP) complexes.

A specific class of lncRNAs, known as SINEUPs, has been found to specifically enhance the mRNA target translation at post transcriptional level. SINEUPs are named after the transposable element they contain, the inverted mouse SINE (Short Interspersed Nuclear Element) B2 element that acts as effector domains to promote the up-regulation of target mRNAs through an antisense region that overlaps with the target mRNA.

Although SINEUPs have been studied in a wide range of both therapeutic and biological applications, the SINEUP-associated RNP complexes and the way they regulate translation are poorly understood. Here, we focus on the identity, function and structure dynamics of SINEUP-associated RNP complexes during protein translation using an interdisciplinary approach that combines functional genomics and structural biology. To isolate SINEUP-RNP complexes during translation we combined ribosome fractionation with a novel purification technique, MS2-tagged RNA affinity purification (MS2-BioTRAP). Using this approach, we confirmed that SINEUP interacts not only with the known RNA binding proteins such as HNRNPK and PTBP1 but also with other novel interactor partners including the ribosomal subunits. Moreover, SINEUP-RNA localization in ribosomal fractions suggested its potential implication in the initial phase of translation. Future perspective will include cryo-electron microscopy to decipher the 3D structure of SINEUP-associated RNPs. The combination of these approaches will provide molecular details crucial to unravel SINEUP-dependent regulation of protein translation. Furthermore, this study will contribute to a better understanding of the translation regulation mediated by SINEUPs and more broadly, the gene expression regulation mediated by functional lncRNAs.

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NON-CODING RNA GENES

Unraveling the regulatory landscape: RNA-chromatin interactions during human monocyte to macrophage differentiation

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Recent studies in the field of RNA biology have clarified that RNAs undertake a variety of diverse functions beyond carrying genetic information from DNA to protein. Emerging evidence suggests that RNAs, especially long non-coding RNAs (lncRNAs), play pivotal roles in regulating gene expression and chromatin organization by interacting with other RNAs, proteins, or chromatin. However, the full extent of these interactions and their impact on chromatin regulation, especially within the dynamic context of cellular differentiation, remains largely unexplored.

Here we map genome-wide RNA-chromatin interactions in a time course of differentiation from human mocytes into macrophages.

Alongside numerous interactions involving sequences derived from the introns of protein-coding genes, we observed a significant increase in lncRNA interactions across differentiation time points.

Furthermore, differential lncRNA-chromatin interactions were significantly enriched in loci hosting differential cis-regulatory elements (CREs), including the promoters of key macrophage-related genes. These targets harbored binding sites for transcription factors (TFs) with defining roles in macrophage biology.

Our results demonstrate the crucial role of lncRNAs in driving the differentiation of monocytes into macrophages, where their interactions modulate the expression of target genes, partially via TF-mediated mechanisms.

We anticipate these results to provide functional links between lncRNAs and their targets in a genome-wide and dynamic context, and shed light on their diverse regulatory mechanisms of action. Integration with 3D chromatin conformation datasets will be essential in characterizing the lncRNA-chromatin interactions mediated by spatial proximity and linking them to functional DNA loops (e.g. promoter-enhancer hubs). In addition, binding data for selected TFs will help elucidate the physical or functional links between TFs and lncRNAs. Finally, analysis of the interactions originating from the intronic regions of protein coding genes will uncover their potential non-coding regulatory roles, akin to those of known lncRNAs, expanding the repertoire of regulatory RNAs.

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RARE DISEASES

A PATHOGENIC VARIANT IN THE KDM3B JUMONJI-C DOMAIN: FAMILIAL OCCURRENCE OF DIETS-JONGMANS SYNDROME

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KDM3B gene is located on chromosome 5q31 and encodes KDM3B, which is involved in histone demethylation and epigenetic regulation. Pathogenic KDM3B variants cause a dominantly inherited disorder presenting with intellectual disability, short stature, and facial dysmorphism, named Diets-Jongmans syndrome. We describe a family with a pathogenic KDM3B variant in two patients, brother and sister, inherited from the paucisymptomatic mother. The two siblings share phenotypic features including short stature, intellectual disability, joint hypermobility, and characteristic facial features such as a wide mouth, a pointed chin, long ears, and a low columella, leading to a Diets-Jongmans syndrome diagnosis. Most of the pathogenic variants in the KDM3B gene are missense and truncating variants, spanning the three zinc-finger, LXLL motif, and Jumonji-C domains, thereby suggesting that haploinsufficiency is the underlying mechanism for this syndrome, but the specific biological consequences and molecular mechanisms are still unknown. In this study, we evaluated the pathogenic missense variant NM_016604 c.5156AG (p.Glu1719Gly) together with a selected set of known heterozygous pathogenic missense variants of KDM3B in-silico. We first ran twenty pathogenicity predictors and summarized their scores. We then used MetaDome to calculate mutation tolerance at each position in the KDM3B protein and assessed the AlphaMissense prediction for them. Finally, we obtained the 3D model of KDM3B from the AlphaFold protein structure database and mutated it in-silico with ChimeraX to introduce all variants. Models were neutralized, energy-minimized, and equilibrated prior to simulation with a Gaussian accelerated molecular dynamics technique. Each system was simulated three times using AMBER 20 on the Leonardo petascale supercomputer. The simulated trajectories of all mutant and wild-type proteins were analyzed comparatively in terms of atomic root mean square deviation profiles, dynamic cross-correlation maps, and the potential of mean force profiles. Long-range effects were additionally investigated to disclose potential pathogenic effects on critical KDM3B protein regions, not evident by studying the sole localization of the variations in the protein structure nor from energetic studies conducted on rigid in-silico models. Hence, genotype-phenotype correlations were introduced according to the degree of similarity of the computed mutants' dynamic properties and patients' phenotypes.

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RARE DISEASES

Identification of multiple de novo variants in newborn with brain malformations: A case report

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We report the case of a 3-months-old girl brought to our attention with a phenotype characterized by spastic hypertonia, axial hypotonia and facial dysmorphism (frontal bossing, low-set ears, micrognathia). A brain MRI showed ventriculomegaly and corpus callosum hypoplasia. No family history of congenital malformations. She was born from spontaneous conception by caesarean section at 41 weeks of gestation with Apgar score 5/9.

Array-CGH and conventional karyotype were normal. Trio NGS in-silico panel was performed using ClinEX pro kit (4bases) on the NovaSeq6000 platform (Illumina), focusing on genes related to the clinical query of cerebral marfomation. NGS analysis showed four heterozygous de novo pathogenic or likely pathogenic variants in PPP2R5D, EEF1A2, CHD4 e CHD7 genes. All genes are involved with brain`s development of neurological pathways.

Pathogenic variants in these genes are associated to autosomal dominant disorders: Houge-Janssens syndrome (MRD35, OMIM #616355 - PPP2R5D gene), epileptic encephalopathy and intellectual developmental disorder (DEE33, OMIM #602959; MRD38, OMIM #616393 - EEF1A2 gene), Sifrim-Hitz-Weiss syndrome (OMIM #617159 – CHD4 gene) and CHARGE syndrome (OMIM #214800 - CHD7 gene).

To date, at 1 year-old, she has addictional clinical features: cleft uvula, dysphagia, laryngomalacia, epileptiform anomalies, severe obstructive sleep apnea and cortical anomalies on MRI.

Next-generation sequencing approaches allowed us to obtain a more complete view of the potentially genes implicated in the phenotype and highlights the importance of genomic data analysis. The variants identified in the four genes suggest oligogenic mechanisms that contribute to the complex clinical phenotype and bringing about new questions regarding the role of each mutation on current clinical manifestations and on phenotype evolution.

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PANGENOMES AND GENOMIC DIVERSITY

A COMPREHENSIVE PICTURE OF THE GENOMIC 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 three major centres of agriculture invention and cattle domestication in the Fertile Crescent. While an "Iranian Neolithic component" is currently recognized in most of Eurasia because of past migrations, 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 genomic diversity and history of the Iranian populations, we conducted a novel WGS study (depth 30×) of 87 males spanning 11 different populations and 3 language families (Indo- European, Afro-Asiatic and Turkic). After merging our WGS data with the relevant available modern and ancient genomes, and considering Y chromosome and mitogenome diversity, we have found that all present-day Iranians, regardless of ethnicity, have two major genomic components: a predominantly pre-Neolithic Iranian autochthonous ancestry and a significant (although variable) ancestry from the Near East/Anatolia introduced into Iran since the Late Neolithic.

The genomic impact of Bronze Age western Steppe pastoralists is also clearly detectable since 4,000 years ago and is evident in all the present-day ethnicities, although its association with the introduction of the Indo-European languages in Iran remains disputed. Despite sharing these three major genomic ancestries, different Iranian ethnic groups also showed striking differences due to varying gene flow from sub-Saharan Africa (Arabs and southern Iranians), central/eastern Asia (mainly in Iranian Turkic- speakers Turkmen, Qashqai and Azeri), and South Asia (in Indo-European speakers of southern Iran). Uniparental marker diversity supported autosomal inferences, but also revealed that gene flow from Africa into Iranian Arabs (but not into southern Persian) was sex-biased.

Our results not only shed light on the differential history of Iranian ethnicities, but also may have biomedical implications. Our study produced the first large scale collection of Iranian complete genomes, contributing to fill a gap in the scarce Middle East representation in variome world-wide collections. This would allow the creation of a population-specific reference genome that could increase imputation accuracy in SNP-based genome-wide association studies as well as gene identification in family studies.

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ETHICAL, LEGAL AND SOCIAL ISSUES ON HUMAN GENETICS

THE HUGO CLINICAL GENOMICS EDUCATIONAL SURVEY: A GLOBAL ASSESSMENT OF PHYSICIANS' KNOWLEDGE OF THE APPLICATIONS OF GENOMICS

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In November 2022, the HUGO Education Committee launched a broad survey designed to measure physicians' other health care providers' perceptions, concerns, and educational needs pertaining to the clinical applications of genomic medicine. A primary goal of the exploratory survey was to understand the gaps in knowledge and preferences for different styles of educational programs across non-geneticist physicians worldwide. The education committee will use survey data to design and deliver future programs to physicians that may accelerate the use of genomic data in health care practice. Twenty-five voluntary questions were asked using the Qualtrics™ online survey tool covering four general topics 1) Demographics/Descriptors: medical specialty, location of practice, type of healthcare system, and past genetics education; 2) Application of Genomics: relevance to their practice, need for genetics in their specialty; 3) Familiarity with Genomics: current knowledge, comfort using or discussing topics, use of clinical guidelines, and available local genomic resources; and 4) Education need and desire: clinical genomics topics of interest; learning style preferences, and need for medical education curricular updates or revisions. Over 300 individuals from 17 countries and representing over 20 medical specialties provided survey responses; 93% of individuals reported participation in genetics education in their past. Remarkably, 97% of the respondents report practicing in Low- or Middle-Income countries, by World Bank classifications, so the data collected provides important information distinct from similar data collected and published from high-income and better resourced medical education and healthcare systems. Survey responses document that physicians around the world are eager to expand their knowledge and utilize genomics in their practices. To this end the HUGO Education Committee will use data collected data and future survey work to inform the creation of new training resources.

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RARE DISEASES

LONG-READ WHOLE GENOME SEQUENCING IDENTIFIES HIDDEN PALINDROMIC INSERTIONS IN A FAMILY WITH X-LINKED CONGENITAL ATAXIA.

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Long-read techniques have complementary strengths to overcome the limitations of current standard genomic technologies, with a particular advantage in analyzing complex or repetitive genomic regions and identifying structural variants. By using Long-Read Whole Genome Sequencing (lrWGS), we aimed to identify the underlying pathogenic cause of a non-syndromic X-linked congenital ataxia with evidence of cerebellar atrophy at neuroimaging studies. In a large family of Norwegian descent, linkage to Xq25-q27.1 region was determined (Zanni et al 2008; (SCAX5 OMIM# 300703) but the underlying causal variant remained elusive despite all routine assessments including short-read genome sequencing (srWGS). Long-read sequencing analysis detected a complex genomic rearrangement including an inverted interstitial insertion of chromosomes 7p14.3 (99 kb) and 9q34.3 (160 kb) within the human-specific palindrome located in Xq27.1, which is known to be a hotspot for genomic rearrangements. Breakpoint junctions segregated with affected individuals in the family. To test the possible consequences of the disruption of topologically associating domains (TADs) combined with other positional or dosage effects, on the expression of cerebellar-specific genes located within the Xq27.1 palindromic region, we performed qPCR analysis on patient-derived lymphoblastoid cell lines and controls. Cerebellar degeneration protein 1 (CDR1), located downstream the palindromic rearrangement and Protein Phosphatase 1 Regulatory Subunit 17 (PPP1R17), present in the inserted 7p14.3 fragment, both specifically involved in cerebellar Purkinje cell development, were found to be upregulated in patients' lymphoblastoid cell lines. Our study highlights the added value of assembly-based lrWGS to detect new genomic disorders and provide insights into the pathogenesis of a previously undiagnosed rare condition. Acknowledgments: This work is dedicated to the Memory of Professor John M. Opitz who followed-up the family for many decades and Dr G Neuhäuser who first reported the pedigree in 1975. We thank all family members for participating in the study.

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GENETICS OF INFECTIOUS DISEASES

PREVALENCE OF HELMINTHIC INFECTIONS AND VARIATION IN HUMAN IMMUNE RESPONSE GENES IN THE INDIGENOUS ORANG ASLI COMMUNITY FROM PENINSULAR MALAYSIA.

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Soil-transmitted helminth (STH) infections affect approximately 3.5 billion individuals worldwide, predominantly in tropical and subtropical regions, particularly in underdeveloped and developing countries. Hookworms, roundworms, and whipworms are the primary helminth species infecting humans, typically transmitted through oral-faecal route or skin penetration. Classified as neglected tropical diseases by the Centre for Disease Control and Prevention (CDC) and World Health Organization (WHO), these infections disproportionately impact the indigenous populations such as the Orang Asli (OA), who reside on peninsular Malaysia in Southeast Asia, due to their lifestyle and limited infrastructure. The relationship between urbanization levels and helminthic infections in three OA subtribes: a rural (Jahai), a semi-urban (Temiar), and an urban (Temuan) population was examined. Results based on examination of 98 faecal samples revealed an overall helminthic prevalence of 50.7%, with Trichuris trichiura (8.08%) being most prevalent followed by Ascaris lumbricoides (1.16%). The Jahai community exhibited the highest helminthic burden, followed by the Temiar, while the urbanized Temuan had the lowest burden and statistically backed using Kruskal-Wallis test (p-value of 0.00071). Whole exome sequencing of Jahai (n = 30) and Temuan (n = 40) DNA identified 71 variants in the curated IL gene list using Ensembl Variant Effect Predictor (VEP) tool. This tool also highlighted three high-impact variants in interleukin (IL) genes (IL4, IL7, and IL34). An additional five population specific variants in IL1F10, IL16, IL17A, IL23A, and IL37 were found using Fine Mapping of Adaptive Variation (FineMAV). IL1F10 had the highest FineMAV score in the Jahai population whereas IL34 had the highest FineMAV score in the Temuan population. IL1F10 suppresses the production of pro-inflammatory cytokines which showcases its role in preventing excessive inflammation and maintaining homeostasis. Polymorphisms within IL1F10 were reported to result in inflammatory diseases such as arthritis, autoimmune diseases, and variations in inflammatory markers such as C reaction protein. IL34 promotes differentiation and viability of macrophages and monocytes. Dysregulation of this cytokine can cause immune-inflammatory disorders. These preliminary findings shed light on OA health and genetics, laying the groundwork for more detailed studies on neglected tropical diseases in these underrepresented indigenous communities in Southeast Asia.

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RARE DISEASES

PHENOCOPY OF WILLIAMS SYNDROME DUE TO PATHOGENIC TBR1 VARIANTS: A REPRESENTATIVE CASE AND REVIEW OF THE LITERATURE.

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Williams syndrome (WS) is a rare genetic disorder characterized by neurodevelopmental delay (NDD)/intellectual disability (ID), typical behavioural profile, growth delay, cardiovascular disease, connective tissue abnormalities, and distinctive facies.

The condition is usually due to a heterozygous 1.5- to 1.8-Mb deletion of the critical chromosomal region 7q11.23. Nevertheless, a subset of individuals reaching the WS diagnostic criteria remains undiagnosed. Several genes are currently under evaluation as causative of WS-like phenotypes.

We describe a 5-year-old female affected by NDD/ID with typical behavioural anomalies (e.g. hypersocial behaviour, hyperacusis) and distinctive dysmorphic features (i.e., broad forehead, bitemporal narrowing, epicanthal fold, periorbital fullness, stellate iris pattern, short nose, broad nasal tip, malar flattering, full cheeks, long philtrum, thick vermilion of the upper and lower lips and wide mouth). No major malformation was noted. WS was clinically suspected without chromosomal microarray (CMA) confirmation. Whole exome sequencing revealed a likely pathogenic variant affecting the TBR1 gene, known to cause a spectrum of neurodevelopmental disorders with behavioural abnormalities collectively termed IDDAS. To date, no recognizable craniofacial profile had been reported for IDDAS.

Through a systematic review of 25 affected individuals and iconographic data of 12 patients, a recurrent and recognizable craniofacial appearance associated with IDDAS, resembling WS, was identified. This includes features such as a broad forehead (11/13), bitemporal narrowing (7/13), strabismus (8/13), epicanthal folds (7/13), short nose (6/13), broad nasal tip (9/13), malar flattening (7/13), full cheeks (9/13), long philtrum (10/13), wide mouth (6/13), and large ear lobes (7/13). Furthermore, behavioural abnormalities observed in IDDAS significantly overlap with those of WS. Both conditions exhibit connective tissue involvement/joint hypermobility. On the other hand, no major malformations nor cardiovascular disease have been reported in IDDAS to date.

These findings suggest the existence of a phenocopy of Williams syndrome, referred to as IDDAS, which may be considered as a potential differential diagnosis for CMA negative WS. The study aims to gather additional cases to further characterize IDDAS and, eventually, establish clinically relevant genotype-phenotype correlations.

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RARE DISEASES

A LYRM7 SPLICING VARIANT IN A CASE OF INFANT DEATHS IN AN ITALIAN FAMILY

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Autosomal recessive mitochondrial complex III deficiency is a rare disorder of oxidative phosphorylation characterised by typical neuroradiological and clinical features. It can be caused by biallelic mutations in several different nuclear-encoded genes. Among these, LYRM7 plays a critical role in the final stages of mitochondrial complex III assembly, acting as a chaperone for the Rieske iron-sulphur (Fe-S) protein in the mitochondrial matrix. We report the case of a 31-year-old woman seeking preconception counselling who mentioned of a brother and a sister who died of unexplained causes at ages of 10 months and 5 years, respectively. The two children were reported to have had peculiar features such as kinky and fragile hair. In the girl the diagnosis of leukoencephalopathy had been made. Both died after an infectious event. Exome sequencing performed on DNA extracted from the consultant`s blood revealed a heterozygous likely pathogenic splice variant (c.91+1GC, NM_181705.4) in intron 2 of LYRM7 gene. The c.91+1GC variation is located in a canonical donor splice site and is predicted to cause exon skipping, shortening, or inclusion of intronic material. mRNA studies are ongoing. The c.91+1GC LYRM7 change was found as a single copy in both parents, suggesting a possible consanguinity by genetic isolation, considering that they come from the same small Italian village (Auletta 2120 citizens). The c.91+1GC, as well as other pathogenic changes in LYRM7, were not identified in the consultant's partner, who comes from the same geographical area. In conclusion, based on the molecular findings and the available clinical features, we could infer that the two deceased brothers were homozygous for the LYRM7 c.91+1GC variant. Therefore, the exome analyses allowed the couple to be properly counseled regarding their reproductive risk for the reported pathology. The present case highlights the clinical utility of identifying the causal variant(s) in those healthy relatives of individuals who have died from a severe undiagnosed childhood onset disorder and who have a high probability of being carriers. De facto, the provision of at least a highly presumptive diagnostic label and the assessment of the reproductive risk may favourably influence reproductive decisions.

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REPRODUCTIVE GENETICS

CONNECTING THE DOTS OF ENDOMETRIOSIS DISEASE: THE ROLE OF GENETICS, DIET, AND MICROBIOME.

Aurora Santin[1], Alessandro Pecori[2], Beatrice Spedicati[1,2], Anna Morgan[2], Stefania Lenarduzzi[2], Paola Tesolin[2], Giuseppe Giovanni Nardone[1], Silvia Camarda[1], Harry Stevens[1], Giulia Pianigiani[2], Lara Emily Rosso[1], Elena Vinerbi[3], Cristina Bon[2], Daniela Mazzà[2], Giovanni Di Lorenzo[2], Federico Romano[2], Francesca Buonomo[2], Alessandro Mangogna[2], Giuseppina Campisciano[2], Gabriella Zito[2], Serena Sanna[3,4], Maria Pina Concas[2], Giuseppe Ricci[1,2], Giorgia Girotto[1,2]

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Introduction:

Endometriosis (EM) is a multifactorial gynaecological disorder where genetics, microbiome, and diet are emerging as key players; however, little is known about their impact on its etiopathogenesis.

This study aims to 1) detect rare variants within EM-known genes and new players, deepen the relationship between 2) microbiome, 3) diet, and EM-symptoms severity.

Methods:

A deeply characterised cohort of 98 EM patients was enrolled at "Burlo-Garofolo" hospital (Italy). Further, EM patients filled in an EPIC-food frequency and a food-preferences questionnaire. For patients that underwent surgery (n=49), vaginal/rectal swabs were collected for microbiome analyses.

1) In comparison to PMID:37626618, WES was performed in additional 18 patients to detect rare damaging variants.

Regarding 2) and 3), associations between EM-symptoms severity, microbiome, and diet were analysed with regression models on the surgery cohort. EPIC-items were grouped according to PMID:33432175, and food preferences-items into categories, comprising high-FODMAP, Dairy, and Gluten-containing foods. Results were compared with 47 controls (Women4Health-cohort).

Results:

1) WES identified 78 rare damaging heterozygous variants within 26 genes. In the extended cohort, a) other two patients carrying variants, respectively, within SYNE1, SYNE2 genes, reported severe dysmenorrhea, consistent with previous findings; b) a patient with EM-related infertility carries a variant within TYK2 gene, already associated with this clinical outcome.

2) A reduced rectal alpha-diversity was associated with increased premenstrual (p=0.013) and ovulation pain severity (p=0.012). A lower premenstrual pain severity was observed with Eubacterium hallii (p=0.01) and Holdemania (p=0.008) genus presence, both known to exert anti-inflammatory properties.

3) Mediterranean diet vegetables consumption was associated with a lower a) premenstrual pain (p=0.0003), and b) dyschezia (p=0.015) severity, thus supporting the role of this food category in ameliorating pain/inflammation.

Finally, high-FODMAP (p=0.009), Dairy (p=0.006), Gluten-foods (p=0.009), and Alcoholic beverages (p=0.03) preferences were detected as possible risk factors, thus highlighting the potential role of these food categories in EM onset.

Conclusions:

This study highlighted the unexplored role of rare variants in EM and provided novel insights on the debated relationships between dysbiosis, diet and EM-symptoms severity, thus paving the way for novel therapeutic strategies and personalised nutritional plans development aimed at improving patients' quality of life.

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GENETICS OF INFECTIOUS DISEASES

Genetic diversity of the Ig heavy chain locus in SARS-CoV-2

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This study was designed to explore the association between a genomic region relevant for B-cell maturation and antibody production and the outcomes of the Covid-19 infection.

This study is dictated by the early observation that antibody production in SARS-CoV-2 is highly variable, in terms of Ig class and timing of seroconversion.

The Ig heavy chain locus includes the gene segments encoding for the carboxy termini of different Ig classes as well as two enhancers named 3'RR1 and 3'RR2. The central portion of 3'RR1 is polymorphic in length with strong disequilibrium with flanking variants. Variation in this genomic region has implications on interindividual diversity in the ability to make Ig switches. The polymorphisms located in the HS1.2 surrounding region (IgHA2, IgHE, IgHG4, IgHG2, 3'RR1, IgHA1) are poorly considered in the literature, while evidence is accumulating on the importance of HS1.2 as an enhancer in model organisms.

We genotyped for 5 SNPs three cohorts of subjects:

A) Patients admitted and treated at the Microbiology and Virology unit of University Hospital, classified as Severe (N = 111) (suffering from respiratory failure, requiring invasive ventilation and intensive care unit admission) or Moderate (N = 182) (showing respiratory impairment, requiring non-invasive ventilation and continuous positive airway pressure or bilevel positive airway pressure cycles).

B) Resistors (N = 139 unvaccinated individuals who, despite repeated exposure to the virus, did not become infected). This category showed strong excess of young subjects (= 50).

C) Control subjects (N = 204) matched for population affiliation, as represented by in-house controls and Tuscans of the 1KG project.

Our results show that:

- No significant differences were observed between sexes in any of the status category.
- The resistor cohort differed only marginally from control subjects.
- The two closest markers that flank HS1.2 centromerically and telomerically displayed significant shifts of allele frequencies in the cohort of severe patients as compared to resistors; this points to HS1.2 as a strong candidate to drive the observed association.
- Four markers displayed frequency trends according to severity.

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RARE DISEASES

EVALUATION OF THE AMPLIDEX ASSAY FOR THE DIAGNOSIS OF FRAGILE X SYNDROME IN MALES AT PUBLIC MEDICAL GENETICS SERVICE

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Fragile X Syndrome (FXS) is one of the most common causes of inherited intellectual disability and occurs in individuals with CGG repeat expansion in the 5'UTR region of the FMR1 gene (Xq27.3), affecting primarily males. Molecular diagnosis of FXS is challenging because the full mutation comprises more than 200 CGG repeats. Since 2009, a repeat primed FMR1 PCR assay (AmplideX) has provided the detection of large full mutations and resolution to evaluate female heterozygosity. In 2020, this assay became available in the Molecular Laboratory of the Medical Genetics Service at the Hospital de Clínicas de Porto Alegre, a 40-year public health hospital in Southern Brazil. This study aims to present the main results of the first four years using the AmplideX assay to diagnose FXS at this laboratory. We reviewed the 2020-2023 molecular reports of FXS diagnosis, which include a PCR-electrophoresis screening test followed by the AmplideX assay for the individuals with positive or inconclusive screening test results. For all individuals with a negative AmplideX result, the reports of the Chromosomal Microarray Analysis (CMA) were also assessed. From 2020-2023, 363 male patients underwent FXS molecular diagnosis; n=25/363 (6.89%) had positive screening results and were forwarded to the AmplideX assay, which confirmed a full mutation in eight patients (2.2%). Other three patients (0.8%) were diagnosed with a pre-mutation (55-200 CGG repeats) and one patient (0.27%) had an intermediate (45-54 CGG repeats) allele. Five of the eight patients with the full mutation had mosaicism (1.3%). Considering the 338 patients with a negative screening test, 105 were forwarded to CMA; of these, n=57/105 already have a result available. Seven patients were diagnosed with chromosome deletion syndromes and the other seven patients have a copy number variation of unknown significance. In conclusion, the AmplideX assay provided the diagnosis of eight patients with FXS (2.2% of the cases evaluated) and the CMA confirmed chromosomal syndromes in the other seven patients (1.9%). In perspective, the detection of female heterozygosity by AmplideX and the clinical profile of the patients diagnosed with FXS will be evaluated.

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GENETICS OF COMPLEX DISEASES

METABOLOME AND WHOLE EXOME SEQUENCING DATA INTEGRATION TO STRATIFY METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE PATIENTS

Rebecca Filomena[1], Carla Debernardi[1], Cecilia Di Primio[1], Angelo Savoca[1], Gian Paolo Caviglia[2], Chiara Catalano[1], Clara Viberti[1], Elisabetta Casalone[1], Chiara Rosso[2], Angelo Armandi[2], Alessandra Allione[1], Marcello Manfredi[3], Miriam Rosselli[1], Elton Jalis Herman[1], Alessia Russo[1], Elisabetta Bugianesi[2], Giuseppe Matullo[1,4]

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Background/Objectives: Metabolic dysfunction-associated steatotic liver disease (MASLD) is defined as the accumulation of excessive fat in the liver in the absence of excessive alcohol consumption or other liver diseases. It is the most common cause of liver disease worldwide and includes a wide range of liver damage, such as simple steatosis (metabolic dysfunction-associated steatotic liver; MASL) and metabolic dysfunction-associated steatohepatitis (MASH). The aim of this study is to find a potential correlation between the genetic and metabolic component of the disease than can better differentiate the phenotypes based on the severity.

Methods: We performed untargeted metabolomics analysis and Whole Exome Sequencing (WES) on 154 patients with biopsy-proven MASLD status (46 MASL and 108 MASH), enrolled at the Liver Unit of the Department of Medical Sciences, University of Turin.

Results: From the untargeted metabolomics analysis, 8 metabolites out of 380 resulted to be significantly differentially expressed (p-value0.05, 0.769Fold Change1.3). Pathway analysis showed that all of them are involved in the Aminoacyl-tRNA biosynthesis pathway (FDR=6.82×10−5).

By combing these results and exonic variants, through a metabolite-QTL, we selected the exome variants based on frequency (MAF0.4) and deep coverage 20. We observed 3 non-synonymous variants (MAF0.03) in HS6ST3, IRF2BPL and MAP3K10 genes significantly associated to metabolites levels, all annotated as variants of unknown significance. These genes are involved in well-known biological process correlated with MASLD progression such as fibrosis, insulin resistance, liver inflammation as well as lipid metabolism.

Conclusion: The integration of metabolomics and WES could provide new insights into the mechanisms involved in MASLD development. Moreover, this combination could result in a non-invasive diagnostic approach for the distinct stages of MASLD, improve patients' stratification and potentially develop new therapeutic options.

Grants: Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) Project "Dipartimenti di Eccellenza 2018–2022. Project n° D15D18000410001".

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GENETICS OF COMPLEX DISEASES

A TRANSCRIPTOME META-ANALYSIS OF CARBAMAZEPINE EXPOSURE IN HUMAN EMBRYONIC CELL LINES

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Carbamazepine is a first-line antiepileptic drug that can be used in the treatment of other neurological and psychiatric disorders. There are controversies regarding the association of carbamazepine use in pregnancy and major congenital anomalies; some cohort studies described an increased risk for prematurity, low birth weight, facial dysmorphisms, developmental delay, and neural tube defects. Other studies presented children with mild phenotypes that might be related to other antiepileptic drugs used simultaneously. Understanding the impact of carbamazepine in the gene expression might help to better comprehend its effects on embryo and fetal development. Hence, this study aimed to perform a meta-analysis of publicly available transcriptomes of human embryonic cells exposed to carbamazepine to assess the genes differentially expressed when compared to control samples. To accomplish this, the Gene Expression Omnibus (GEO) database was assessed. Only studies of carbamazepine exposure, without other drugs, in human embryonic cells were included. RNA-seq samples were processed in the useGalaxy server. Differential gene expression (DGE) was calculated in R v.4.2., using the affy package for microarrays and the edgeR package for RNA-seq. Meta-analysis was also performed in R with the metavolcanoR package, using the Fisher-P method, that is indicated to reduce false positive results in transcriptome analysis. Genes differentially expressed across the studies (metagenes) were considered significant if | meta-logFC 1 | and P-Value adjusted for false discovery rate 0.05. Five datasets (GSE126786, GSE209962, GSE187001, GSE69395, and GSE64123) were selected and processed. The meta-analysis provided only four metagenes: DKK4, H1-4, FBP1, and CELP; none of them have been previously associated with carbamazepine. H1-4, a histone gene previously associated with craniofacial dysmorphisms, was the only upregulated in carbamazepine exposure of the four metagenes presented. CELP is a pseudogene and FBP1 encodes for a gluconeogenesis enzyme. DKK4 encodes for a protein member of the dickkopf family, with high expression in osteoblasts and a role in embryonic development through the Wnt signaling pathway. The association between carbamazepine and the metagenes here presented must be further investigated in order to comprehend whether the alterations in the genes' expression might impact on embryo and fetal development.

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RARE DISEASES

Investigating Pleural Mesothelioma Susceptibility: Integrating Proteomics into Risk Assessment

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Objectives: Pleural Mesothelioma (PM) is an aggressive, rare form of cancer of the pleural compartment which is caused by exposure to asbestos and related minerals. The disease is difficult to detect early due to its asymptomatic long latency period and advanced-stage diagnosis. Currently, there is no set way to diagnose or treat PM and thus it is imperative to discover predictive early-stage biomarkers to improve the diagnosis and prognosis.

Methods: Our research aimed to study the proteomic landscape found in the serum samples of 21 PM prospective patients and 21 asbestos-exposed controls from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. We used a high-resolution SWATH-MS technology to analyse the proteins and further verified the results with an ELISA assay.

Results: A total of 324 proteins were quantified, of which 12 were seen to be differentially expressed (p-value 0.05, FC 1.3 and 0.7) between PM and asbestos-exposed control subjects less than 5 years before PM diagnosis. A panel of significantly identified proteins was subsequently validated and replicated on a subgroup of Molecular Markers for early detection of cancer (MoMar) cohort using the ELISA assay. We observed a significant increase in prospective cases of Transferrin (TF) and Complement C4A (CO4A), while b2-microglobulin (B2M) and Dermcidin (DCD) decreased. Furthermore, we also validated the already known Mesothelin and Calretinin biomarkers in our EPIC cohort as well.

Conclusion: This study provides us an insight on significant proteins that could potentially assist in diagnosing PM. It emphasized the importance of exploring the bio-fluids proteome to identify potential biomarkers in a time-dependent and sensitive manner. This approach could potentially enable early identification of individuals exposed to asbestos who are at high risk of developing mesothelioma, leading to better understanding of causation and discovery of new treatment targets. However, further validation in larger sample size is necessary. Integrating these novel proteomic biomarkers with existing ones like mesothelin and calretinin could enhance sensitivity and facilitate the development of a panel of biomarkers for PM diagnosis. Moreover, we will soon integrate exome sequencing and proteomics data to investigate genomic alterations at the protein level and genetic susceptibility.

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GENETICS OF COMPLEX DISEASES

GENOMIC INSIGHTS INTO PARKINSON'S DISEASE OF A LARGE ITALIAN COHORT

Agata Fant[1], Francesco Musacchia[1], Sara Trova[1], Andrea Gaudio[2], Fabio Landuzzi[1], Gaia Treves[1], Luca Pandolfini[3], Edoardo Monfrini[4], Alessio Di Fonzo[5], Lucia Trevisan[6], Andrea Cavalli[1], Marco Di Giovanni[7], Nicoletta Lillia[7], Laura Caligiana[7], Susanna Cordera[7], Guido Giardini[7], Manuela Vecchi[1], Paola Mandich[2], Stefano Gustincich[1]

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Objectives

Parkinson's disease (PD) is heterogeneous both clinically and genetically with complex phenotypes and different progressive courses. In addition to recent advancements on the identification of both rare and common variants contributing to disease onset and risk, a comprehensive evaluation of the whole genome landscape in subjects with PD will improve our knowledge of the genomic basis of this disease.

The study aims at deciphering the genetic impact of coding and non-coding regions of genomes of PD patients in Northern Italy and at integrating whole genome sequencing (WGS) in clinical practice.

Methods

WGS is conducted on PD subjects with short-read (Illumina) technology. Additionally, for selected cases, long-read (Oxford Nanopore) sequencing is also performed. Bioinformatic analysis is carried out using the GPUs-accelerated NVIDIA Clara™ Parabricks® automatized pipeline. We also developed a consensus pipeline for Structural Variants (SVs) analyses.

Results

We have carried out WGS of several hundreds of PD patients on the Illumina platform and long-read sequencing on a selected group of subjects. In a subset of cases, we have identified pathogenic and risk variants in known causative genes, and candidate variants in non-coding regions of the genome. We have also identified Copy Number Variants that haven't been detected with standard clinical methods.

Conclusions

The integration of short- and long-read sequencing allows the identification of novel mutations and mechanisms/pathways in unsolved clinical cases with PD. Overall, this study demonstrates that incorporating WGS in clinical practice allows the detection of diagnostic variants that conventional methods failed to identify.

POSTER SESSION 3
APRIL 09
FROM 16:15 TO 17:15

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PRECISION HEALTH

THE 5000GENOMI@VdA PROJECT: AN ITALIAN WHOLE GENOME SEQUENCING INITIATIVE

Manuela Vecchi[1], Alessandro Coppe[1], Fabio Landuzzi[1], Sara Trova[1], Andrea Cavalli[1], Stefano Gustincich[1]
CMP3VdA, Italian Institute of Technology, Italy

Background

The 5000genomi@VdA Project is a unique Italian initiative funded by the Autonomous Region Valle d'Aosta, in the Northwest of Italy. The project is carried out by a multidisciplinary consortium led by the Italian Institute of Technology. It takes advantage of collaborating closely with the Valle d'Aosta healthcare system and other regional research centers/hospitals in Italy.

Objectives

Since analyzing the entire genome is not a standard practice in clinical genomics studies, in this project short- and long-read whole genome sequencing (WGS) approaches are employed to study coding and non-coding genomic regions.

The Project aims to sequence 5,000 genomes of healthy donors and patients with neurodevelopmental, neurodegenerative, oncological, and organ transplantation diseases.

Through the synergistic interaction between private-public research and healthcare centers, the goal is to study the role our genes play in health and disease conditions, thus promoting the integration of WGS in the clinical domain.

Methods

Genomic DNA is derived from whole blood and, in cancer patients, also from tumor and non-pathological tissues. We currently sequence samples with short-read WGS using the Illumina NovaSeq 6000 platform (coverage 40x for blood and 100x for tumor samples). Bioinformatic analysis is based on GPU-compliant and accelerated pipelines (NVIDIA Clara™ Parabricks®) implemented in the computational genomics infrastructure of the CMP3VdA center. We also perform long-read sequencing in selected cases using the PromethION device (Oxford Nanopore Technologies).

Results

We developed the genomic and computational infrastructure to analyze and store large-scale genomics data sets. So far, we have performed more than 2,000 whole genome sequences with Illumina. We have also produced long-read sequencing data on selected cases as a complementary approach to accelerate research discovery and improve diagnostic rates. RNA-seq has also been carried out for specific individuals.

Additionally, genomic analysis of healthy blood donors enables the characterization of the Valle d`Aosta Region's population genetic diversity and genetic architecture.

Conclusions

This study represents a strategic blueprint healthcare model for implementing WGS into clinical practice in Italy.

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CANCER GENOMICS

WHOLE EXOME SEQUENCING (WES) AND SHALLOW WHOLE GENOME SEQUENCING (SWGS) OF TISSUE AND CELL-FREE DNA (CF-DNA) FOR THE ASSESSMENT OF HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD) IN OVARIAN CANCER

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Background: pathogenetic variants in the genes BRCA1/BRCA2 have a significant role in developing ovarian cancer (OC) and are essential for genome integrity by repairing double-stranded DNA breaks via the homologous recombination repair (HRR) pathway. Our aim is to identify whether the HR disruption correlates with oncogene alterations and, ideally, with therapy responses.

Methods: a total of 96 women affected by OC (13 in paclitaxel-platinum with or without bevacizumab, 3 in PARP-inhibitors) were enrolled. We performed: on 66 samples a multigene panel on FFPE tissue and HRS-test to identify HRD; on 30 samples WES (germinal and somatic) and SNP-array; on all 96 samples shallow whole-genome sequencing (sWGS), which results were compared to the reference assay SOPHiA. Furthermore, sWGS was also performed on cf-DNA in 50 cases.

Results: we found germinal variants in BRCA1/BRCA2 on 5.21% and 6.25% of subjects, respectively. Moreover, we detected several variants in suppressor genes and oncogenes, including mismatch repair (MMR) genes, likewise causative variants in HRR-related genes like BARD1 (2,08%), ATM (2,08%), CHEK2 (1,04%). Somatic pathogenic variants were: 71,88% in TP53, 16,67% in BRCA1 and 11,46% in BRCA2. Almost all cases (98%) presented genomic instability highlighted both on tissue and cf-DNA.

Conclusions: BRCA1/BRCA2 mutation status is the main genetic biomarker of HRD but is not sufficient to describe the total HRD status, which can be driven by somatic events and further genetic alterations. Therefore, a bioinformatics pipeline to evaluate the HRR system status in OC has been set, based on sWGS, to support therapeutics and follow-up strategies.

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RARE DISEASES

MULTIPLE ONLINE RESOURCES CREATED TO FACILITATE GLOBAL GENETICS & GENOMICS EDUCATION

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INTRODUCTION:
There is a growing, well-recognised, requirement for access to genetic and genomic educational resources for professionals, students, teachers & the public, worldwide.

OBJECTIVES
To facilitate and enhance worldwide cutting-edge human genomics education and training, at all levels.

METHODOLOGY
The author (winner of the ESHG Education Award 2021) has created and made available an expanding range of online educational resources. These now include: (1) a free easily-navigated web-based guide, to a range of international genetic and genomic educational sources: ESHG Genetic Educational Materials and Sources (www.EuroGEMS.org) for all audience levels, now in English, Spanish, Portuguese and French. To facilitate its use, its pages are now linked from the HUGO website; (2) iOS and Android smartphone clinical genomics apps, for students and professionals worldwide, explaining clinical genomics and basic bioinformatics terminology and permitting self-assessment. (3) a new set of free gamified online genomics quizzes; (4) a set of FutureLearn massive open online courses (MOOCs) on clinical genetics, medical genomics and cancer genomics, co-led with Glasgow University colleagues; and (5) custom-designed software (compatible with the protein databank) for virtual reality (VR) headsets, to facilitate immersive 3D visualisation of (wild-type and variant) protein molecular structure.

RESULTS
The author's www.EuroGEMS.org educational genomics website has now been accessed from a total of 138 countries. Senior professionals' comments include: "an excellent, comprehensive and highly useful resource for education". The smartphone genomics apps have now been used by 5000 people in 70 countries and have been rated 5* on iOS & Android app stores. They are easy to use and can be accessed and downloaded, free of charge, via www.genomicsapps.org. The MOOCs have now been used by 50,000 learners from 115 countries.

CONCLUSIONS
It is anticipated that all the above (and other) online educational resources will be used increasingly by professionals, students and the public, worldwide. Efforts are currently underway to increase access to them. The author, a UK academic clinical geneticist who runs undergraduate and highly-popular Masters-level medical genetics courses, is an active member of the ESHG, ECMGG and HUGO Education Committees. He continues to develop the resources and would welcome feedback and suggestions at: edward.tobias@glasgow.ac.uk.

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REPRODUCTIVE GENETICS

GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND ITS IMPACTS ON THE EMBRYONIC DEVELOPMENT

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Glucose-6-phosphate dehydrogenase (G6PD) plays a role in housekeeping and has an immense role in maintaining the redox system of the body. It also facilitates de novo synthesis of nucleotides which are essential for the rapidly dividing cells during embryonic development. The pathway catalysed by G6PD is the source of reducing equivalent nicotinamide adenine dinucleotide phosphate (NADP) required to generate reduced glutathione which is essential in the phase II detoxification of xenobiotics. Thus, alterations in the gene leading to relatively reduced protein expressions could have deleterious effects either in isolation or in association with other proteins resulting in birth defects. Hematological effects resulting from this enzymopathy have been well characterized over the years but little is known concerning birth defects. To verify the potential risk factor(s) of G6PD deficiency for the onset of congenital anomalies, we evaluated the proteins that interact with G6PD through computational analyses. We consulted Expression Atlas to verify G6PD gene expression during embryonic development. In sequence, we employed the PINOT tool to obtain the interacting proteins and performed a Gene Ontology (GO) overrepresentation analysis through clusterProfileR package in R v.4.3.2. We found that G6PD is highly expressed in several organs, such as the liver, brain, testis, heart, kidney, and ovary from the fourth-week post-conception (wpc). In the heart, expression is diminished from the 10 wpc, and in the liver, the expression is high until the neonate stage, being reduced only after birth. In the other organs evaluated, high expression is maintained throughout the whole life. PINOT results presented 31 proteins that interact with G6PD. The GO analysis revealed that these proteins are related to processes in early development, such as gastrulation, primary germ layer formation, and endoderm development and differentiation. Consequently, our results revealed the early elevated expression of G6PD during embryonic development. Although further analyses are required to confirm or refute the hypothesis that G6PD deficiency might be a risk factor for congenital anomalies, it is well established that increased oxidative stress harms the embryo. Hence, G6PD could act as a protective factor to maintain the redox status during prenatal development.

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RARE DISEASES

UNEXPECTED TRYPTOPHAN-KYNURENINE PATHWAY METABOLITE PROFILE IN A CHILD CARRYING DOUBLE DE NOVO COPY NUMBER VARIANTS

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A 6 y.o. female presenting with neurodevelopmental delay, absence of language, dysmorphic features, bilateral hypoacusia, congenital malformations and attention deficit hyperactivity disorder, was referred for genetic counseling. The proband was born at 36 GW +3 with cesarean section for oligohydramnios, after delivery baby resuscitation was needed (Apgar 5-8-10).
SNParray analysis through CytoSNP-850K platform on genomic DNA extracted from peripheral blood revealed 2 de novo Copy Number Variants: 1q41q42.12 microdeletion spanning almost 3.2 Mb and 7q21.11q21.12 microdeletion spanning almost 2.4 Mb.
1q41-q42 deletion (MIM#612530) is characterized by congenital multiple anomalies, developmental delay, and dysmorphic recognizable features. It includes 50 genes, whose the major candidate critical gene for contributing to patients' phenotype is WDR26. WDR26 haploinsufficiency has been suggested as the cause of Skraban-Deardorff syndrome (MIM #617616).
In patient's lymphocytes and fibroblasts a significant decrease in expression levels of WDR26 was confirmed by qPCR and most patient' features are consistent with the reported phenotype.
7q21.11 microdeletion involves 20 genes; few patients are reported in literature with larger deletions.
To further explore possible related-pathophysiological mechanisms, a liquid chromatography/tandem mass spectrometry (LC/MS-MS) was required to quantify whole-blood metabolites in a research setting. Impaired levels of major metabolites in the Tryptophan-Kynurenine metabolic pathway and NAD levels reduction were evidenced. This pathway generates essential neuroactive metabolites in the Central Nervous System and it has recently been involved in the complex pathogenesis of Autism Spectrum Disorder. The initial and rate-limiting step is catalyzed by either indolamine 2,3-dioxygenase (IDO1 and IDO2) or tryptophan 2,3-dioxygenase (TDO).
RNAseq performed on patient's fibroblasts disclosed a very low expression of IDO1, IDO2, and TDO. Expression analysis by qPCR confirmed the decrease of IDO1 and TDO transcript levels, both in the patient`s fibroblasts and lymphocytes compared to a healthy control. Western Blot analysis on patient`s fibroblasts highlighted also a decrease in IDO1 and TDO protein levels.
LC/MS-MS performed after one year in new acquired samples, confirmed the Tryptophan-Kynurenine metabolites alteration in patient' serum.

Integrative analysis of DNA Methylation and microRNA are ongoing to identify a common regulator of these enzymes.
Multi-omics approach could unravel unexpected pathways dysregulation, possible clues to explore therapeutic targets.

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PANGENOMES AND GENOMIC DIVERSITY

ESTABLISHMENT OF A NATIONAL RESOURCE OF POPULATION AND DISEASE-ASSOCIATED GENETIC VARIABILITY IN SLOVENIANS

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Characterisation of a nation's genetic architecture is the basis for the provision of accurate genetic testing, implementation of genetic screening programmes and development of novel disease risk prediction models on a national scale. In the recent years, population sequencing efforts have been undertaken in larger, particularly Anglo-Saxon, populations. However, several populations have been significantly under-represented in these projects, including the genome of the Slovenian population. To address this, we aggregated sequencing data from 8.025 Slovenian individuals to establish a resource of genetic variability of Slovenians.

We aggregated exome (ES) and genome sequencing (GS) data from two cohorts: 7.566 individuals with ES and 459 with GS data. Raw data were re-processed and variants called jointly to the hg38 reference genome observing the GATK best practice guidelines. Strict variant quality filters were applied to the call set and only variant sites that were accurately genotyped in over 90% of either the ES or GS cohort were considered to represent accurate estimations of variant frequencies. The variants were annotated using the Variant Effect Predictor and stored in a local SQL database and collected in an accessible web browser.

Altogether, 26.439.624 distinct variants were identified in the dataset, of which the majority (96.3%) were detected in the GS cohort. Of the variants identified, 3.070.774 (11.6%) were not observed in the gnomAD 2.1.1 project, indicating that they represent novel variation of the human genome. We also observed significant differences in variant frequencies, with 8.3% variants (MAF0.01%) displaying over two-fold differences in allele frequencies when compared with global gnomAD populations. These differences were particularly prominent for ClinVar variants, where 77.0% of pathogenic variants observed in our population were either absent from or deviated significantly in frequency from gnomAD populations.

In conclusion, we established a resource of genetic variability of Slovenians. The results expand the knowledge of genetic variability in Slovenians and this region of Europe. Given the observed distinctiveness from gnomAD populations commonly used in genetic data interpretation, we also anticipate this resource to facilitate diagnostic and research efforts in our population.

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COMPUTATIONAL BIOLOGY AND AI

REVOLUTIONIZING PRECISION HEALTH: UNVEILING THE SYNERGY OF MEDICAL IMAGING AND GENETIC INTEGRATION ANALYSIS WITH ARTIFICIAL INTELLIGENCE

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The convergence of medical imaging and genetic data within expansive biobanks, propelled by artificial intelligence, is reshaping precision health. This research delves into the realm of precision health by scrutinizing abdominal ultrasonography and bone density scan images, in conjunction with whole-genome single nucleotide polymorphisms, to assess Type 2 Diabetes risk in a cohort of 17,785 Han Chinese participants from the Taiwan Biobank. Employing convolutional-network deep-learning models for imaging analysis and statistical-learning models for genetic analysis, we fuse these modalities utilizing the machine-learning method. Our findings from imaging analysis underscore the superiority of pixel-based analysis over feature-centric methods in terms of accuracy. Furthermore, the incorporation of multi-modality analysis enhances accuracy compared to single-modality approaches. When combined with medical imaging, genetic, and demographic information, these strides open up promising avenues for fusion modeling through artificial intelligence to refine disease risk assessment. The ultimate model yields a compelling area under the Receiving Operation Curve of 0.944 in accurately assessing the risk of Type 2 Diabetes. This methodology significantly advances our comprehension of precision health in the context of Type 2 Diabetes.

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GENETICS OF COMPLEX DISEASES

CORRELATION OF PRS WITH CELL TYPE COMPOSITION AFTER ILR TRANSFORMATION PROVIDES NEW INSIGHTS ON THE RELATIONSHIP BETWEEN IMMUNE-RELATED DISEASES AND PBMCs CELL TYPE PROPORTIONS

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Several diseases present inflammatory or immune-related dysregulation which can be reflected in an unbalanced cell type composition in peripheral blood mononuclear cells (PBMCs). Here, we use polygenic risk score (PRS) of immune-related phenotypes, and ILR transformed cell-type proportions to investigate the relationship between immune-related phenotypes and cell type composition at the single cell level. In this study, we re-analyzed data from the Onek1K dataset (Yazar et al, 2022), containing genotyping data and single-cell RNA-seq data for 982 individuals. We applied the most recent best practices for single-cell quality control and performed cell type annotation using Azimuth and Celltypist tools. We then manually curated cell groups based on marker genes and cell labels to obtain a precise representation of cell proportions in each individual and to construct a hierarchical tree of cell groups. To mitigate statistical challenges related to compositional data, we applied Isometric Log-Ratio (ILR) transformation to represent proportions with new unbiased ILR directions. The PRS of each individual was then computed using LDPred2 for the trait of interest and correlated to these new ILR directions. To assess the validity of our method, we first applied this method to two cellular phenotypes, namely monocyte counts and lymphocyte counts. This showed the expected results with PRS positively and negatively correlated, respectively, to ILR directions representing the ratio between monocyte and lymphocyte. We then applied the same approach using PRS based on GWAS summary statistics obtained from UK Biobank (UKBB) or PGS catalog for several immune-related traits, including Inflammatory Bowel Disease (IBD), Ulcerative Colitis, Crohn's Disease, Atopic Eczema, Asthma, Type 1 Diabetes Mellitus (T1DM) and body mass index (BMI). Our preliminary results showed significant correlations for IBD, T1DM and BMI with dimensions related to specific cell groups, providing possible new insights on the relationship between immune-related phenotypes and cell composition at the single cell level.

In summary, our approach provides a novel and robust method to correlate a phenotype of interest and cell-type composition. The initial findings about the influence of immune-trait PRS can give new insights into the intricate relationship between genetic predisposition to inflammatory traits and immune dysregulation.

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CANCER GENOMICS

PAEDIATRIC MELANOCYTIC TUMOURS: UTILITY OF A -OMIC APPROACH

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Spitz lesions are a spectrum of melanocytic proliferations, i.e. Spitz nevi (SN), atypical Spitz tumours (AST) and Spitz melanomas (SM), showing an overall favorable behavior. AST and SM share histological features with aggressive adult-type melanomas, making their distinction challenging.

We evaluated the impact of a -omic approach on diagnostic accuracy of paediatric melanocytic lesions.

The series encompassed 45 Spitz lesions (13 SN, 24 AST, 8 SM), 1 atypical congenital nevus (ACN), 3 paediatric melanomas, 2 melanomas arising in giant congenital melanocytic nevi (GCMN) and, as control group, 7 adult melanomas. RNAseq, SNP/CGH array and NGS variant panel were performed in 57, 43 and 12 cases, respectively.

Kinase genes were altered in 11/13 NS, 21/24 AST, 6/8 SM, confirming Spitz signature in 84% of cases: fusion involving ALK (13 cases), MAP3K8 (6), RET (5), ROS (4), MET (2), NTRK1 (3), NTRK3 (3), and LCK (1) and HRAS mutation (1). Multiple segmental chromosomal alterations (msCNA) were identified in 2/8 NS (including a case of HRAS mutated-desmoplastic SN with 11q amplification), 5/18 AST, 5/5 SM, involving a melanoma risk locus (6q loss) in 1 AST. No kinase-genes alterations were found in 7/45 Spitz lesions. Among those, a SM showed NF1 inactivation and CDKN2A homozygous deletion qualifying the lesion as adult-type melanoma; another SM displayed a novel MTPAP::TCF7L2 fusion, suggesting an adult-type melanoma. The remaining 5 lesions need further investigation.

Concerning non-Spitz lesions, the ACN showed a MAP3K8 fusion and chromosome 12 msCNA, leading to its reclassification as an AST. Pediatric melanomas showed the signature of adult-type melanomas: mutation in BRAF and TERT promoter (1 case), NRAS (2 cases) and msCNA, namely CDKN2A homozygous deletion (2 cases), and 6q loss and 8q gain (1 case). In the 2 cases of melanomas arising in GCMN, we identified NRAS mutation in both the malignant and GCMN components (VAF 0.5 and 0.2 respectively), and msCNA exclusively in the melanoma components.

The -omic approach i) documented the Spitz signature in 38/45 (84%) Spitz lesions and in 1 ACN thus re-interpreted as AST, ii) re-classified 2 SM as adult-type melanoma. Five Spitz lesions lacking Spitz signature need further investigation.

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CANCER GENOMICS

IMPACT OF RARE AND LOW-FREQUENCY GENETIC VARIANTS IN PLEURAL MESOTHELIOMA

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Background. Previous Genome Wide Association Studies (GWASs) in pleural mesothelioma (PM) identified low risk genetic variants conferring a limited increase risk of the diseases and showing evidence of interaction with asbestos exposure. Other sequencing studies on cancer gene panels in PM cases identified 5-10% of germline mutations increasing susceptibility to PM even at low levels of asbestos exposure. Here, we conducted a whole exome sequencing (WES) analysis aimed at identifying genes with rare variants contributing to PM risk.

Methods. WES screening was performed on DNA isolated from blood of 129 pre-clinical PM and 129 non-cancer individuals. Alignment of the sequencing reads, variant calling and annotation were performed using JuliaOmixTM software (GenomeUp, Italy). SNVs with a read depth 20X and a m.a.f. 0.01 in GnomAD database, were taken into account for downstream analyses.

We developed a gene-level collapsing model to identify potential less penetrant risk variants in the coding region with a potential clinical impact on the disease development.

Results. In the 3% of all individuals, we identified pathogenic rare variants in cancer predisposing genes BRCA2, ATM and BAP1, which are reportedly associated with PM, as well as mutations in RB1, CIC, MCAM, whose involvement in the disease is still unknown.

The gene-level collapsing test was performed in 10-fold cross-validation with 80% of samples in each run, considering 112000 variants. The analysis showed an enrichment of rare variants in several tumor suppressor genes involved in cancer invasion and cell division.

Conclusions. WES sequencing technologies allowed us to identify rare germline mutations in novel genes potentially contributing to an increasing risk of PM development, together with asbestos exposure. Our study paves the way for further analyses to investigate the combined impact of rare variants in genes involved in PM onset and broaden the mutational landscape of the malignancy.

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REPRODUCTIVE GENETICS

PRENATAL IDENTIFICATION OF REA(21Q21Q) BY QF-PCR AND STANDARD KARYOTYPE IN A FETUS WITH INCREASED NUCHAL TRANSLUCENCY AND MATERNAL ABNORMAL SERUM SCREENING.

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Down syndrome (DS) is the most frequent chromosomal aberration at birth and is determined by the triplicate state (trisomy) of chromosome 21 critical regions. Only in 2% of cases, DS is due to 21q;21q rearrangements [rea(21q21q)].

Here we report a 36-year-old primigravida presenting with a high risk of fetal trisomy 21 based on nuchal translucency (3,6 mm) and maternal serum biochemical markers (free β-hCG: 64,6 UI/l; PAPP-A: 0.480 UI/l) in the absence of structural abnormalities visible on fetal ultrasound.

Quantitative fluorescent polymerase chain reaction (QF-PCR) using a set of STR markers for chromosomes 13, 18, 21, X, and Y was performed on DNA extracted from uncultured amniotic fluid cells collected at 17 gestational weeks and cytogenetic G-band analysis was performed on cultured amniocytes and parental peripheral blood lymphocytes.

QF-PCR screening showed a normal male chromosome pattern and a normal diallelic pattern for chromosomes 13 and 18. In contrast, chromosome 21 revealed a trisomic diallelic pattern. Standard karyotyping confirmed the presence of 3 copies of chromosome 21 and revealed one normal chromosome 21 and one isochromosome for the long arm of chromosome 21 in all cells examined (46,XY,i(21)(q10)). The pattern indicated a homologous duplication of chromosome 21q which is likely due to a division error that occurred during meiosis II or postzygotic mitosis. De novo origin can be reasonably assumed since parental chromosomal analysis came out normal, although the existence of germ-line cells mosaicism could not be ruled out.

Isochromosomes from identical chromosome arms [i(21q)] account for the vast majority of rea(21q21q) and need to be distinguished from robertsonian translocations between two different chromosome 21 arms [der(21;21)(q10;q10)]. Isochromosomes, in fact, originate from postzygotic events of centromere misdivision or U-type strand exchange between sister chromatids and are associated with reduced recurrence risk. This report substantiates the critical role of prenatal cytogenetic analysis in the diagnosis of fetal chromosomal aberrations and subsequent reproductive risk assessment.

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GENETICS OF INFECTIOUS DISEASES

T CELL EPITOPE MAPPING OF LEPTOSPIRAL OUTER MEMBRANE PROTEIN FOR VACCINE DEVELOPMENT

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Leptospirosis, a zoonotic disease caused by the spirochete bacteria Leptospira, affects both humans and animals. Despite recent research efforts, the pathophysiological mechanisms of Leptospirosis remain incompletely understood. Symptoms in humans can range from mild flu-like symptoms to severe and potentially fatal conditions. The absence of a commercially viable vaccine for Leptospirosis has led to significant public health challenges, particularly in areas where the disease is endemic. Developing an effective vaccine has garnered considerable attention, with epitope-based vaccination emerging as a promising strategy for identifying potential candidates.

This research aims to identify an epitope-based vaccine candidate within pathogenic and intermediate Leptospira species using both in silico and in vitro methodologies. Through comprehensive in silico analysis of Leptospira proteins, potential vaccine candidates with crucial immunogenicity are identified. Various bioinformatics techniques are employed to predict conserved regions associated with potential epitopes. LipL46, a selected outer membrane protein, is found to be conserved among 43 pathogenic and 21 intermediate Leptospira species, indicating its stability. Ten conserved regions are identified through multiple sequence alignment tools, with three regions (88-105, 288-297, and 317-328) selected for further analysis based on criteria such as conservancy, antigenicity, and molecular docking.

A shortlist of candidates is prepared through in silico screening, which is then validated in vitro using indirect ELISA with archived human serum samples from patients with Leptospirosis. Among the candidates tested, vaccine candidate 3 demonstrates higher potential antigenicity and immunogenicity compared to candidates 1 and 2. However, further in vivo studies are necessary to confirm the safety and efficacy of the epitope-based vaccine candidate selection narrowed down in this study.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

Comparative evaluation of long and short read sequencing technologies for human trio genotyping

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Recent advances in sequencing technologies have paved the way for personalized medicine and clinical genomics, providing unprecedented insights into the genetic basis of human health and disease. The application of high-throughput genome scanning is now recognized as an effective first-tier diagnostic tool for patients affected by rare diseases but the use of either Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS) technologies has shown to be uninformative in about 50% of the cases. This is due to both technical limitations and to the nature of genomic lesions underlying the disease. On the contrary, the recent introduction of long read technologies can resolve genomic regions inaccessible to short-read sequencing, and may represent a breakthrough. Thus, a reliable benchmark assessment is mandatory to assess the real impact of long read technologies in upgrading clinical practice.

This study presents a comparative evaluation of long-read and short-read sequencing data, aiming to elucidate their respective strengths and limitations in the context of genotyping and clinical genomics applications. Moreover, it has been performed through the investigation of family pedigrees, which represents a powerful approach for the identification of transmitted alleles and/or de novo mutations that may confer susceptibility to the disease.

We deeply investigated a family trio with a pediatric proband affected by a rare disease for which WGS analysis based on short reads resulted uninformative for the diagnostic assessment. The trio comparative assessment presented here has provided a high-confidence assessment of short and long read performances in variant calling accuracy, coverage uniformity, detection of structural variants, and allele specificity. Long-read technologies, known for their ability to span repetitive regions and capture structural variations, are evaluated for their impact on genotyping accuracy and the identification of complex genomic rearrangements.

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GENETICS OF COMPLEX DISEASES

INVESTIGATION OF PEDIATRIC MULTIPLE SCLEROSIS BY APPLYING MULTI OMICS DATA INTEGRATION

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Multiple Sclerosis (MS) is a chronic inflammatory multifactorial disease that occurs between 20 and 40 years of age. Only 5% of MS patients develop the disease before 18 years, so we focused on the pediatric form of the disease (PedMS) that offers a unique opportunity to gain clinical, biological, environmental information in proximity to the actual disease onset. Our study aimed to identify genetic and environmental components associated to PedMS by performing multi omics data integration.

After recruiting an Italian multicentric cohort, we performed GWAS (Illumina GSA and HRC imputation), metagenomic (16S sequencing) and methylation (Illumina Epic Array v2) analyses on PedMS (up to 460) and matched healthy controls (HC, up to 1693). We integrated data following either an early strategy with MOFA (omics matrices are concatenated in a single one containing features from all omics for co-occurrent

samples) and a late one (each omics is analysed independently, and the most significant results are merged in the end).

We performed a preliminary late integration of the most significantly associated data from GWAS (1916 SNPs with p1x10-5) and 1382 differentially methylated regions from epigenetic analyses, identifying an overlap between 14 SNPs and 4 significant DMRs. In particular, one DMR maps in RNF39 gene and was already described in literature. This gene encodes a protein that exerts E3 ubiquitin ligase and it is close to TRIM family, which has antiviral activity. Moreover, some SNPs in this region are in linkage disequilibrium with one of the 33 HLA MS-associated loci. We also investigated the co-localization of those SNPs and DMRs with 200 MS associated loci known from literature, identifying 2 significant overlaps for each omics.

We have analysed several omics individually underlining differences between PedMS and HC. We have already performed a preliminary late integration analysis identifying an interesting region, and we are combining genetic, epigenetic and metagenomic data by an early and a late integration strategy to identify latent factors that could explain PedMS pathogenesis.

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RARE DISEASES

Variant of Uncertain Significance in RAB23 in a family with Carpenter syndrome associated to defective lateralization

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Introduction. Carpenter syndrome (OMIM 201000) is an ultra-rare autosomal recessive disorder characterized by acrocephaly peculiar facies, soft tissue syndactyly, brachy- or agenesis mesophalangy of the hands and feet, preaxial polydactyly, congenital heart disease, mental retardation, hypogenitalism, and obesity. It is associate to mutations in the RAB23 gene and MEGF8, the latest is associated to Carpenter 2 syndrome which is characterized by defective lateralization in addition to main anomalies. Our aim is to present a new familial case of Carpenter syndrome with defective lateralization and a VUS in RAB23. Patient and Methods: 3-year-old male patient, product of fifth pregnancy from healthy and non-consanguineous parents. Antecedent of a sister with craniosynostosis. Delivered by cesarean section at term, birth weight of 2950g and height of 49cm, head circumference of 46.3 cm. At birth, a congenital cardiopathy was detected (univentricular heart with atresia of the atrio-vertebral apparatus) as well as craniosynostosis. PE: generalized cyanosis, acrocephaly, precordium with presence of a heart sound systolic murmur, bilateral cryptorchidism, brachydactyly of the second fingers at the expense of the middle phalanx and clinodactyly of the second, third and fourth fingers, acropaquia, normal pulses. Abdominal ultrasonography showed situs inversus, with splenomegaly and probable polysplenia. Due to the clinical characteristics, Carpenter syndrome was diagnosed, and a sequence analysis and deletion/duplication testing of the 2 genes MEGF and RAB23 was conducted. Results: An homozygous Variant of Uncertain Significance (VUS), c.317_322del (p.Lys106_Val108delinsIle) was identified in RAB23. This variant, c.317_322del results in the deletion of 3 and insertion of 1 amino acid(s) in the RAB23 protein. It has been classified as a VUS since the available evidence is currently insufficient to determine the role of this variant in disease. A segregation analysis was performed, the similarly affected sister and an unaffected brother were studied. VUS c.317_322del was identified in RAB23 in homozygous state in the affected sister, whereas this variant was not detected in the healthy brother. Therefore, we propose that VUS c.317_322del is likely pathogenic. This is the first report of a familial case of Carpenter syndrome 2 with a likely pathogenic variant in the RAB23 gene.

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PANGENOMES AND GENOMIC DIVERSITY

A HUMAN PAN-GENOMIC ANALYSIS REFINES THE GENETIC LANDSCAPE OF THE FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY LOCUS

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Background and Objectives: Two percent of the genome is composed of tandemly arrayed repeats whose organization, number and arrangement were impossible to interrogate until the advent of long read sequencing technologies.

Facioscapulohumeral muscular dystrophy (FSHD), is a natural example of a hereditary neuromuscular disease whose molecular defect resides in the reduced number of the D4Z4 tandemly arrayed elements at 4q35 subtelomere and offers the possibility to connect the variations of the copy number of repetitive genome with human physiology and disease.

The exact sequence composition of the D4Z4 array has never been explored because of technical limitations due to its repetitive nature. The recent release of the T2T genome assembly and of the Human Pangenome Project dataset (PGR) now give the possibility to delve into these sequences at the single repeated unit level.

Method: We assessed the distribution and composition of D4Z4 elements in T2T and in the 86 haplotype-level genome assemblies from the PGR.

Results: Analysis of the T2T and the PGR assemblies confirmed the presence of hundreds of complete 3.3kb D4Z4-like elements, as well as partial ones, spanning from 0.7 Mb to 1.5Mb of the genome (compared to 86.5Kb in GRCh38). In PGR haplotypes, 4.6% of cases bared 4q alleles with ≤8 D4Z4 units, associated to the 4qA-PAS haplotype permissive for FSHD.

Four principal types of sequences were identified: 2 for the 4q-specific and 2 for the 10q-specific repeated elements. However, the 4q-specific elements exhibited greater variability and abundance, likely attributed to their more ancient origin. The African populations showed low heterogeneity in terms of D4Z4 array composition, bearing a single 4q and a single 10q repeat type, while other populations presented more complex arrays with variable proportions of the different repeat types.

Conclusion: The advent of long-read sequencing technologies, applied to diverse populations, revolutionizes the study of repetitive elements like D4Z4. This breakthrough lays the groundwork for further evolutionary and functional studies, promising insights into individual pathogenic phenotype variability.

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RARE DISEASES

ADVANCING PEDIATRIC GENOMIC MEDICINE: A STRATEGIC IMPLEMENTATION FRAMEWORK FOR RARE DISORDERS IN QATAR

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Introduction:
Genomic medicine has emerged as a transformative approach in healthcare, particularly in the realm of pediatric rare disorders. This abstract outlines the strategic implementation of genomic medicine for addressing rare pediatric disorders in the healthcare landscape of Qatar.

Objective:
The primary objective of this initiative is to establish a comprehensive framework for the integration of genomic medicine in the diagnosis, treatment, and management of pediatric rare disorders in Qatar. The focus is on leveraging cutting-edge genomic technologies to enhance precision in healthcare delivery for the pediatric population.

Methods:
This implementation plan involves the collaboration of multidisciplinary teams, including geneticists, pediatricians, bioinformaticians, and healthcare policymakers. Genomic sequencing technologies, such as whole-exome and whole-genome sequencing, were employed for comprehensive genetic analysis. The initiative also incorporate data sharing mechanisms, ethical considerations, and education programs for healthcare professionals.

Results:
The ongoing and anticipated future outcomes include improved diagnostic accuracy, personalized treatment plans, and enhanced understanding of the genetic basis of rare pediatric disorders in an underestimated region. By integrating genomic data into clinical practice, healthcare providers can make informed decisions tailored to the unique genetic makeup of each patient.

Conclusion:
This abstract presents a strategic roadmap for the implementation of genomic medicine to address pediatric rare disorders in Qatar. The initiative aims to propel precision healthcare forward, ultimately improving the quality of life for children affected by rare genetic conditions. The insights gained from this implementation may serve as a model for other regions seeking to integrate genomic medicine into their healthcare systems.

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GENETICS OF COMPLEX DISEASES

INVESTIGATION OF ENVIRONMENTAL AND GENETIC FACTORS ASSOCIATED WITH BRUXISM IN FIVE GENETICALLY ISOLATED POPULATIONS FROM NORTH-EASTERN ITALY

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Introduction

Bruxism is a repetitive activity of the jaw muscles characterized by clenching or grinding the teeth. It is a widespread multifactorial disease worldwide, but little is known about its genetic component.

Aim

The aim of this study was to test possible risk factors associated with bruxism and identify new genes potentially associated with this disease through a Genome-Wide Association Study (GWAS).

Methods

Bruxism was diagnosed during a thorough odontostomatological examination that assessed the presence of tooth wear and self-reports in a cohort of 769 people (age 6-89 years) from five genetically isolated villages in North-Eastern Italy (FVG Genetic Park).

Logistic mixed models (LMM) adjusted for age and sex were performed to evaluate associations between bruxism, and selected possible risk factors such as anxiety, depression, smoking, alcohol, and caffeine intake. A case-control GWAS was performed on all subjects with available genotyping data (135 cases, 523 controls) using a logistic model adjusted for anxiety, sex, age, village of origin, and the first ten principal components. GTEx data analysis was carried out to evaluate the identified genes expression in human body tissues.

Results

The LMM revealed that only anxiety was a statistically significant risk factor for bruxism (Odds Ratio 2.55; 95% CI 1.21 – 5.39). GWAS results showed 55 SNPs within 11 genes reaching a suggestive p-value (p10−5). The most interesting findings were: RIMBP2 (14 SNPs with p10−5), NLGN1 (6 SNPs with p10−5) and LHFP (6 SNPs with p10−5) genes. In particular, RIMBP2 (topSNP:rs571497947, p=4.83x10-7) encodes a pre-synaptic binding protein involved in neuromuscular synaptic transmission (PMID:32867148), NLGN1 (topSNP:rs2046718, p=2.54x10-7) plays a critical role in regulating synapses development, transmission, and plasticity (PMID:33522967), and LHFP (topSNP:rs2324342, p=7.47x10-6) encodes a member of the lipoma HMGIC fusion partner protein, which is a tetraspan transmembrane protein firstly identified in lipomas (PMID: 10329012). GTEx data analysis confirmed their expression in brain tissues.

Conclusion

This study confirmed that anxiety is associated with bruxism. GWAS results led to the identification of three promising new candidate genes; considering their relevance in neurobiological mechanisms, these genes could be considered as new molecular players underlying the etiopathogenesis

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PRECISION HEALTH

IMPLEMENTING GENOMIC SEQUENCING AND GENETIC RISK SCORES IN QATARI NEWBORN SCREENING--A PRECISION HEALTH APPROACH--

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Introduction:
The integration of genomic sequencing and genetic risk scores into newborn screening programs has the potential to revolutionize early disease detection and intervention. This abstract explores the implementation of these advanced technologies in the context of Qatari newborn screening, emphasizing a precision health approach.

Objective:
This initiative aims to enhance the effectiveness of newborn screening in Qatar by incorporating genomic sequencing and genetic risk scores. The objective is to identify genetic markers associated with various health conditions, allowing for early detection, personalized risk assessment, and targeted interventions.

Methods:
The implementation strategy involves collaboration between geneticists, pediatricians, and public health officials. Genomic sequencing technologies, coupled with the calculation of genetic risk scores, will be employed to analyze the newborns` genetic information. The approach considers a diverse range of genetic factors, contributing to a comprehensive understanding of potential health risks.

Results:
Anticipated outcomes include improved accuracy in identifying genetic predispositions to diseases, enabling proactive healthcare measures for affected individuals. By integrating genomic data and risk scores into newborn screening, this precision health approach strives to reduce the burden of inherited conditions and enhance long-term health outcomes.

Conclusion:
This abstract highlights the significance of implementing genomic sequencing and genetic risk scores in Qatari newborn screening as a means of advancing precision health. The initiative not only presents an opportunity to identify and address potential health risks early in life but also sets the stage for a more personalized and targeted healthcare approach for the Qatari population. The insights gained from this implementation may contribute to the evolving landscape of newborn screening practices globally, emphasizing the role of genomics in shaping the future of pediatric healthcare.

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RARE DISEASES

TANC2 DE NOVO MISSENSE VARIANT IN A PATIENT WITH AUTISM SPECTRUM DISORDER AND MODERATE INTELLECTUAL DISABILITY

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TANC2-related neurodevelopmental syndrome, also termed IDDALDS (Intellectual developmental disorder with autistic features and language delay, with or without seizures, MIM #618906), is a distinct neurodevelopmental disorder caused by pathogenic heterozygous germline variants in TANC2 gene on chromosome 17q23. IDDALDS was first described by Guo et al. (2019) in 20 unrelated patients with Autism Spectrum Disorder (ASD), Intellectual Disability (ID), speech and motor delay and variable facial dysmorphisms. Here we report on a 5-year-old boy referred to our Genetic Unit and presenting with ASD and moderate ID. He is the first child of healthy non-consanguineous parents with unremarkable family history. He was born at 36 weeks after an uneventful twin pregnancy, his dizygotic twin is in good general condition. The patient walked without support at 18 months and spoke his first words at 24 months, with slow language development. Electroencephalogram (EEG), brain Magnetic Resonance Imaging (MRI), spectroscopy (MRSI), audiometric testing and ophthalmologic evaluation were normal. His parents reported a sleep disorder, for which he is taking melatonin daily. He also presents bilateral pes planus, treated with orthotics. On clinical evaluation, at 4 years and 7 months, his height was 113 cm (1,10 SDS), his weight was 18 kg (-0,19 SDS) and his occipitofrontal circumference was 53 cm (1,88 SDS). He presented high forehead, hypertelorism, epicanthus, thick helices, broad nasal tip, anteverted nostrils, long smooth philtrum, widely spaced teeth, thin upper vermillion and one cafe-au-lait spot. On first-tier we performed G-banded karyotype, fragile-X testing, and Chromosomal Microarray Analysis (CMA), resulted negative. Subsequently we performed trio-based Clinical Exome Sequencing (CES), which detected an heterozygous de novo variant: TANC2(NM_001394998.1):c.4567GA p.(Val1523Ile). This variant is not reported in gnomAD (v4.0) and has been currently classified as of uncertain significance, according to ACMG guidelines. The variant is located in a disordered region of the protein, which could have a regulatory role in the phosphorylation of a downstream Serine (at position 1442), and consequently in TANC2 structure and/or interactions. Functional studies are ongoing and will help to better clarify the role of this variant. We are now recruiting patients with ASD/ID and missense variants in TANC2.

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GENETICS OF INFECTIOUS DISEASES

Long-term effects of HLA A*03:01 genotype on anti-SARS-CoV-2 Spike antibody levels following BNT162b2 vaccine

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Introduction. We and others have recently reported that the remarkable variability of response to vaccines against SARS-CoV-2 is partially determined by the host genetic background. In particular, we found a significant association between the HLA A*03:01 allele and the increased anti-SARS-CoV-2 Spike antibody levels at two months following the first administration of the BNT162b2 vaccine. The present work aims to study the impact of the HLA A*03:01 genotype on antibody titers over time: before and after the booster dose administration.

Material and Methods. From the database of 873 subjects analyzed in our previous study, we selected those with antibody titers measured at 8 and 12-14 months following the first dose of vaccine and with no serological evidence of SARS-CoV-2 infection at the time of antibody measurements. A bootstrapped multivariable piecewise linear mixed-effect regression model was applied to estimate the anti-S decay over time (modelled through a restricted cubic spline) before and after the booster was administered. To test differences among groups, an interaction term between time and the considered groups was introduced.

Results. The analysis of longitudinal data revealed a decrease in antibody titers over time for both individuals carrying the HLA A*03:01 allele and non-carriers. It is noteworthy that subjects with the A*03:01 allele consistently maintained higher antibody titers than non-carriers. The difference between the two groups, however, narrowed as time progressed and it became non-significant about five months after the first dose. The booster dose administration drastically increased antibody levels in both groups, temporarily erasing any differences. However, starting from the 16th week after the booster administration, the differences became evident again. Finally, we confirmed that the effect of smoke on antibody levels remains constant over time and is erased only by booster dose administration.

Conclusion. The booster dose significantly increased antibody levels and reduced the previously observed variability in vaccine response following the primary administration. While the effect of the HLA genotype on antibody titers tends to diminish over time, it remains present even after the booster dose. Regular booster dose administration may be particularly advantageous for particularly categories of individuals.

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CANCER GENOMICS

Rare variants modulate phenotype in NF1 carriers

Rare Variants Modulate Phenotype in NF1 Carriers Giulia Casamassima[1,2], Elena Pasquinelli[1,2], Chiara Fallerini[1,2], Samantha Minetto[1,2], Margherita Baldassarri[1,2,3], Alessandra Reniei[1,2,3]

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Neurofibromatosis type 1, is a rare genetic disorder predisposing to a pleiotropic phenotype ranging from psychosocial issues, congenital malformations, benign tumors of the central and peripheral nervous systems or aggressive cancer in solid organ, such as breast. We hypothesize that this phenotypic variability is due to additional rare variants in other genes, acting as an oligogenic threshold model.

Clinical and Exome sequencing data were retrieved in a retrospective nested-study over a period of three years. We recruited a cohort of 32 subjects with pathogenic/like pathogenic NF1 variant.

NF1 carriers with solid cancers show a mean of 4.12 variants in cancer driver genes per subject while NF1 carriers without solid cancers show 2.16 per subject with Mann-Whitney test significant at p0.05. Gene combination analysis shows that at least one variant (often 2 or more) in DNA repair pathway is present (7/8 cases) in classical (BRCA1, MSH6, POLE, ERCC6, NTHL1, RAD551L3, RAD51C, ATR) and/or FANC genes (FANCL, FANCM, SLX4, FAN1). In addition, one the following, often alternative, pathways is involved: RAS-MAPK (SPRED1, linking to neurofibromin); EGFR/AKT/mTOR (PARK2, SLC6A3, EGFR); BMP/Smad (BMPR1A); PTEN/AKT (PTCH2); NOTCH (FBXW7); WINT/beta catenin (AXIN1), RET (TMEM127), or other function such as histone regulation (NSD1, CREBBP), immune regulation (TNFRSF11A, PTPN12), fibrosis (TERT, MUC5B), integrity of nucleus and Golgi function (SYNE2), and transcription regulation (ETV6).

Furthermore, we show also that less frequently (macrocephaly or cognitive disorder) or rarely (connective disorder or polydactyly) ancillary characteristics could be linked to rare variants in additional genes. NF1 carriers with cognitive impairment have a mean of 2.7 additional variants, with at least one of them being a P/LP variant in a well known dominant neurodevelopmental disorder gene. In inherited cases, we show that NF1 inherited variant combined with other rare variants inherited from both parents act reinforcing the NF1 susceptibility with an additive trend.

In conclusion, we have shown that rare variants may modulate phenotypic outcome in neurofibromatosis. These results are relevant for both clinical policy and clinical practice: we propose to use the rare variant capture for amelioration screening program in NF1 carriers and identification of specific gene cards for personalized treatment.

GENETICS OF COMPLEX DISEASES

Oligogenic inheritance in sporadic Parkinson disease: a retrospective study

Oligogenic inheritance in sporadic Parkinson disea Samantha Minetto[1,2], Giulia Rollo[1,2], Chiara Fallerini[1,2], Elena Pasquinelli[1,2], Anna Maria Pinto[3], Lucia Monti[4], Alessandra Renieri[1,2,3]

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About 15% of Parkinson disease (PD) cases are considered monogenic rare disorders due to one (dominant) or 2 (recessive) mutations in one gene, transmitted following Mendelian rules and called monogenic or Mendelian diseases. However, some hints indicate that in the remaining cases, genetics is likewise relevant, but the number and the nature of genes involved is still unclear. Here we explore the hypothesis that combination of rare variants in a handful of genes is the major etiology and pathophysiology in sporadic PD.

We performed exome-sequencing in a cohort of 14 sporadic PD patients and 26 controls, genetic variants were first prioritized by eVAI tool and then selected on the bases of rarity in both public (0.01 MAF) and internal databases (0.005 MAF) among 68 genes already known to be involved in either PD or Parkinsonisms (PanelApp).

Difference in number of retrieved variants were assessed between patients and controls. Patients had a mean of 3.78 variants while controls 1.84 with Mann-Whitney test significant at 0.0002 (Z score -3.72884).

Gene combination analysis reveals that each patient has at least one rare variant in one of the three areas of PD pathophysiology: endolysosomal/lysosomal function (LRRK2, VPS35, ATP13A2, GBA, LYST, ATP1A3, ATP6AP2, VPS13A), synaptic vesicle trafficking (SYNJ1, RAB39B, SNCAIP, SLC6A3, TH) and mitochondrial impairments (PINK1, HTRA2, PANK2, PLA2G6, c12orf12, COASY), often in addition with one gene related to calcium (PDGFB) or magnesium (SLC30A10) deposits, dopaminergic neuron transcription factor (NR4A2), neuroinflammation (GNR) or axonal transport (TUBB4A, MAPT, or DICTN1).

We have shown that rare variants in PD/Parkinsonism genes are the main genetic determinant in sporadic PD. Considering as marker 3 or more rare variants selected as above, the test has a sensitivity of 0.75 and specificity of 0.86 for PD recognition. This is relevant for clinical policy (identification of patients at risk in the population), clinical practice (identification of specific gene card for personalized treatment, including gene editing) and future research (for better understanding pathophysiological mechanisms).

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CANCER GENOMICS

GENETICS AND GENOMICS OF PANCREATIC CANCER IN ITALIAN PATIENT COHORTS

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Background

Pancreatic ductal adenocarcinoma (PDAC), with a five-year survival rate of less than 10%, is expected to become the second most prevalent cause of cancer-related mortality in both the US and Europe by 2030. About 10% of cases have a family predisposition; however, the heritability of pancreatic cancer may be twice as much. Only 10–20% of patients have resectable disease, but local and distant relapses are frequent. In most cases, conventional therapies such as chemotherapy and immunotherapy fail to provide long-term benefits, underlining the pressing need for innovative approaches to this deadly disease.

Objectives

This research project aims to combine different “omics” (genomics/transcriptomics/epigenomics) to study pancreatic cancer tumorigenesis by using second and third-generation sequencing technologies and patient-derived organoids. This approach will enable the exploitation of cancer vulnerabilities and expand the repertoire of drug targets to the undruggable genome.

Methods

Matched tumor and healthy pancreatic tissues of a retrospective cohort of 58 PDAC patients from the Humanitas biobank were whole-genome sequenced with the Illumina NovaSeq 6000 platform (coverage 100x). Germline and somatic data were analyzed using the GPU-compliant and accelerated bioinformatics pipelines implemented in the computational genomic infrastructure of the CMP3VdA center. We are carrying out long-read-sequencing in selected paired samples using the PromethION device (Oxford Nanopore Technologies).

Results

Interim analysis on the genomic profiling of 45 paired normal-tumor tissues of the PDAC cohort revealed that about 10% of patients had a pathogenic/likely pathogenic (P/LP) actionable germline variant (OncoKB) in genes predisposing to pancreatic cancer (BRCA2, ATM, CHECK2, and MLH1). Additionally, ~14% of patients exhibited P/LP variants in genes predisposing to other types of cancers (PTEN, MSR1, and RNASEL) or hereditary pancreatitis (CFTR), thus raising the overall heritability of pancreatic cancer to more than 20%. The tumor genomic landscape of these 45 cases reflected the frequencies observed for essential somatic alterations (KRAS, TP53, SMAD4, CDKN2A, and GNAS) in different PanCancer cohorts. We are also analyzing structural variations (i.e., chromothripis) to exploit cancer vulnerabilities.

Future perspectives

To expand the number of PDAC Italian patients, we have designed a multicenter national prospective PDAC cohort study.

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RARE DISEASES

MOLECULAR DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE IN AN ITALIAN FAMILY CARRYING RET C.208CT P.(GLN70TER) VARIANT

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Hirschsprung`s disease (HSCR) is an autosomal dominant disorder, with an incidence of 2-2.8 in 10.000 newborns. HSCR is gastrointestinal motility disorder mostly diagnosed in children but it needs to be higher in the differential diagnosis of adults with chronic constipation. HSCR is an enteric neuropathy characterized by bowel obstruction and absence of enteric ganglia in distal colon. Most patients show clinical phenotype in the newborn period. The only treatment for HSCR involves the surgical resection of the aganglionic segment. Pathogenic variants in RET, NRG1, and L1CAM genes have been found in most cases of HSCR. RET proto-oncogene encodes a receptor tyrosine kinase for members of glial cell line-derived neurotrophic factor (GDNF) family. RET is implicated in several inherited human diseases; certain forms of Hirschsprung`s disease are the result of loss-of-function mutations in RET while the cancer syndromes (Multiple endocrine neoplasia) seem to be caused by gain-of-function mutations. RET variants have been found in up to 5% of sporadic HSCR and up to 50% of familial cases. Mutations in RET extracellular domain impair its cell surface expression, most likely due to misfolding of protein. Nonsense changes result in several deleterious forms of truncated proteins, depending on position of variants.

We report a 30 years-old female subject affected by isolated HSCR, who required the surgical total colon resection. Clinical Exome analysis and Sanger sequencing identify a nonsense RET variant, c.208CT p.(Gln70Ter) in exon 2 that causes the loss of the entire RET protein. We extend the analisys to her 1 years-old son who present the same simptoms and required a surgical partial colon resection. The analysis confirms the presence of the variant in heterozygous state.

Our findings suggest a pathogenic effect of this nonsense variant according to others loss of function changes in RET. This variant was previously reported only in just one other study about a cohort of 601 Chinese patients.

We would broad the range of variants associated with HSCR and highlight the importance of molecular diagnosis for prognostic purposes, mostly in pediatric patients with mild phenotype and in familiar cases.

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COMPUTATIONAL BIOLOGY AND AI

REPEATS ENRICHMENT ANALYSIS OF CHIP-SEQ DATASETS (REACH) - HANDLING MULTIMAPPING READS IN CHM13 REPEATS ALIGNMENT

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DNA repeats, non-unique genomic sequences long discarded from in-depth genetic studies, are the predominant source of multimappers. These reads align to multiple genomic locations with identical scores, serving as the main source of ambiguous assignments. No consensus exists on the best multimappers handling approaches, with each strategy pursuing to strike a balance in quantification estimation.

We took advantage of the availability of the telomere-to-telomere CHM13 gapless human genome assembly to construct REACh: our computational pipeline for Repeats Enrichment Analysis from publicly available Chromatin immunoprecipitation sequencing datasets. Initially, we downloaded the RepeatMasker annotation data, generated raw sequence files for each repeat family, and categorized them into four macrofamilies based on their spatial arrangements within the genome: tandem, non-tandem, LINE1 and LINE2. Next, we independently aligned the sequencing reads against each macrofamily in parallel, reporting the top fifty multimapping alignments. We extrapolated repeat-read raw counts by selecting uniquely mapping reads and/or those multimapping on two, and only two, macrofamilies, excluding reads that multimap among the same macrofamily and/or in more than two separate macrofamilies.

However, while the validation process highlighted certain strengths within our method, it underscored the bias associated with non-linearly annotated repeatomes. The RepeatMasker annotation file reveals a vertical stratum of repeats: over four billion base pairs of overlapping, non-unique, shared and nested repeats. Moreover, when the common repeat sequence derives from different repeat families, it also bears divergent annotations converging in the interpenetration site. Hence, we believe that our multimapping exclusion criteria led to oversimplified count quantification and the generation of a reservoir where potentially valid and substantial mappings were ignored.

Therefore, we suggest a tailored multimapping handling strategy, restricting the enrichment comparisons between highly repetitive chromosomal subregions, like those of centromeres and telomeres. This leads to multimappers assignments simplification and reference linearization. Chromosomal repetitive regions are genomically demarcated, with distinguishable features and no interpenetration, resulting in a linear reference without annotative convergence. Thus, after categorising read alignments based on such predefined chromosomal subregions, we set multimapping criteria: reads multimapping within the same region are counted once, while those which multimap to more than one region are disregarded.

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PRECISION HEALTH

A PLATFORM FOR GENOMIC DATA ANALYSIS, INTERPRETATION AND REPORTING

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Whole genome sequencing (WGS) represents a cutting-edge approach to characterising complete genetic profiles of individuals. The management, interpretation and reporting of the expanding genomics data is pivotal. To address this need, we developed a comprehensive platform including data collection, storage, analysis, and automated reporting on an interactive mobile application (app) with downloadable PDF documents, which ensures user-friendly data access and visualisation.

We demonstrate the utility of the platform by analysing and generating reports for the WGS of a newly recruited Turkish (TR) cohort (n=275, 3 with inherited genetic diseases and 272 healthy) and an existing Swedish (SW) cohort (n=101, healthy), as a pilot trial. A total of 35.8 million variants were identified in the TR cohort, including 5.3 million novel variants. The low frequency pathogenic(P) / likely pathogenic (LP) findings were reported based on the ClinVar database for the TR (398 P/LP) and the SW (192 P/LP). We observed that RUNX1 and SMPD1 had the highest carrier frequencies in the TR and SW cohorts, respectively. For the predicted loss-of-function variants (pLoFs) and other risk variants, HLA-DQA1 and HLA-DRB1 regions of genomes were enriched in the TR cohort and CTU2 and SLC52A2 regions of genomes were enriched in the SW cohort. Regarding pharmacogenetic (PGx) variants associated with Tier 1 VIP genes, both TR and SW WGS data included a similar median number of 3 PGx variants with promising evidence levels per sample.The generated reports were compared between the two cohorts, highlighting the genetic heterogeneity of the carrier frequencies of pathogenic variants between the two ethnic groups. These known and novel variants found in the TR cohort and SW cohort were stored on the cloud server for promoting public sharing. The cases of two muscular dystrophy and one microcephaly were investigated through the platform, identifying 3 phenotype-related pathogenic variants.

In conclusion, our platform facilitates WGS data analysis for a wide range of end-users, including clinicians, patients, and individuals undergoing commercial genomic sequencing. Our platform and study provide insights into the genetic characteristics of both TR and SW populations for facilitating public health decisions.

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COMPUTATIONAL BIOLOGY AND AI

Direct inosine detection in native RNA sequences

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Epitranscriptome modifications are now emerging as important factors to fine tune gene expression and regulation. To date more than 170 different RNA chemical modifications have been unveiled up, contributing to the complexity of the gene expression in eukaryotes. Among them, A-to-I RNA editing by ADAR enzymes plays crucial biological roles and has been linked to several human diseases, even though its involvement in disease etiology has not been yet elucidated. A variety of computational tools have been released to profile A-to-I editing through short-reads sequencing. However, distinguishing real editing events from sequencing noise or artifacts is still challenging. In this context, direct-RNA sequencing by nanopores (dRNA) offers the unique possibility to directly identify modifications exploiting alignment glitches and ionic currents perturbations. Available methods to detect inosines via dRNA leverage on computational demanding steps that are independent from basecalling, potentially hampering the real RNA editing landscape as in hyper-edited regions. To fill this gap, we developed the first “inosine-aware” basecaller prototype to natively identify inosines in transcripts from raw currents measurements. More in detail, we have collected, using an in-house software, millions of raw electric signals and their corresponding k-mers starting from dRNA assays of different organisms and in-vitro transcribed synthetic constructs in which the exact locations of inosines were known. Next, a transformer model with an expanded dictionary (A, C, G, U, I) was trained to detect canonical ribonucleotides and inosines harnessing electric measurements only. Preliminary results on an independent set of transcripts from synthetic constructs with or without inosine incorporation, indicate that our basecaller can efficiently identify inosines with a very low amount of false positives. Although further optimizations are needed as well as specific tests on real datasets, our system is unique in detecting inosines during basecalling, providing a puntual map of A-to-I RNA editing at a single molecule level. Remarkably, the direct deciphering of inosines improved the mappability of synthetic transcripts when compared to the same sequences basecalled by state of the art programs recognising canonical bases only.

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REPRODUCTIVE GENETICS

PRENATAL GENETIC COUNSELLING AND REPRODUCTIVE CHOICES IN A MULTIRACIAL AND MULTICULTURAL COMMUNITY: A SIX YEARS' EXPERIENCE IN A MALAYSIAN GENETIC SERVICE.

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Prenatal screening and diagnostic testing had become standard practice in many countries, which help to reduce the birth prevalence and mortality rate secondary to congenital anomalies and genetic disorders. The attitude and acceptance toward prenatal testing and reproductive choice vary with regards to ethnicity, cultural values, religion, health awareness, and educational background of the clients. Whilst prenatal testing is available in Malaysia and termination of pregnancy is legal for maternal indications, these issues are arguably some of the most challenging in a multi-racial and multi-cultural community. We aim to evaluate the outcome and reproductive choice amongst the clients who received prenatal genetic counselling in the genetic clinic, University Malaya Medical Centre (UMMC). We retrospectively review the electronic medical record and identified clients referred and attended genetic clinic for prenatal genetic counselling from June 2016 till June 2022. We then analysed their ethnicity, religion, indication of referral, uptake of prenatal diagnostic testing and reproductive choices. There was a total of 61 clients who received prenatal genetic counselling in the 6 years duration. The indications of referral including previous children with known genetic disorder, previous children with congenital anomalies, parents with a known genetic disorder, the finding of an abnormal antenatal scan, and high-risk non-invasive pregnancy test. The majority of the clients' ethnicity are Malay (n=37, 60.7%) with Islam as their religion. 35 patients (57%) underwent prenatal diagnostic testing and 7 patients (20%) with foetuses affected by a genetic disorder. Five out of the 7 patients (71%) opted for termination of pregnancy. Most of the clients involved their partner (n=54, 89%) in their reproductive decision making. Prenatal genetic counselling by qualified personnel played an important role in shared reproductive decision making. Diversity in the culture and religion were important elements during genetic counselling. There was limited number of clinical geneticists and genetic counsellors available in a resource limited country like Malaysia. Thus, a user-centred, evidence-based patient decision aid that is tailored to each client's culture, religion and needs are recommended

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GENETICS OF COMPLEX DISEASES

INVESTIGATING DIABETIC CARDIOMYOPATHY: A STUDY OF GENETIC AND EPIGENETIC CONTRIBUTIONS

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Background: Diabetic Cardiomyopathy (DCM) develops in a subset of Type-2-Diabetes (T2DM) patients, characterized by cardiac dysfunction independent of ischemic diseases or hypertension. The underlying causes of DCM remain unknown. This study as part of the CARDIATEAM consortium, aims to dissect the genetic and epigenetic landscape of DCM, employing Polygenic Risk Scores (PRS) and Methylation Risk Scores (MRS) for enhanced risk stratification.

Methods: We analyzed 181 clinically characterized participants from a discovery cohort divided into two groups based on the presence of cardiac dysfunction, with a prevalence of T2D among affected individuals. After thorough quality controls, we performed genomic (GWAS) and epigenomic (EWAS) association studies adjusting for Age, Sex, and Genetic Principal Components in order to find potential markers related to DCM related pathways. PRS was performed on the genotype data with effect sizes from a T2D PGS [PGS001357], while MRS employed beta values from 145 CpGs identified in the Generation Scotland Study. We evaluated four logistic regression models incorporating PRS, MRS, their combination and interaction.

Results: Association studies identified genes and epigenetic modifications linked to pathways in cardiovascular diseases, T2DM, and cardiomyopathy. T2D-based PRS effectively differentiated the groups [Kolmogorov P-value 0.02] with significant ORs at higher quantiles, 2.23[95%CI 1.03-4.92] for ≥80th and 2.82[95%CI 1.02-8.34] for ≥90th. MRS findings complement these insights [Kolmogorov P-value 1.29e-05] with OR of 4.52 for ≥80th[95%CI 2.01-10.83] and 3.49 for ≥90th[95%CI 1.22-11.02]. Selecting the individuals with the highest PRS and MRS values, we obtain a significantly higher OR of 6.48 for ≥80th[95%CI 1.81-30.96]. The combined model (PRS+MRS) demonstrated a better discriminative ability (AUC=0.753), whereas the interaction model does not seem to increase the discriminatory power (AUC=0.752).

Conclusion: This study highlights the complex interplay between genetic and epigenetic factors in DCM, paving the way for improved screening and personalized management strategies. Future work aims to validate these interactions and their clinical utility.

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CANCER GENOMICS

WHOLE EXOME SEQUENCING (WES), SNP-ARRAY AND SHALLOW WHOLE GENOME SEQUENCING (SWGS) IDENTIFIES RECURRENT GENOMIC ALTERATIONS AND MOLECULAR HETEROGENEITY IN MULTIPLE MYELOMA.

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Background: multiple myeloma (MM) is a hematologic disease caused by clonal proliferation of plasma cells. We examined the genetic aspects of MM, including sequence-related genetic alterations and genomic instability, to demonstrate the superiority of sWGS, a high-throughput technology, as a valid alternative compared to classical cytogenetics. Indeed, although FISH is the current gold standard to detect cytogenetic events in MM, it is costly, labor-intensive and does not show the exact genomic background of this disorder.
Methods: we analyzed 100 patients at various stages of MM. We performed SNP-array and FISH to detect copy number aberrations (CNA) and balanced translocations, respectively. Additionally, we performed sWGS (0.1X) and WES on each sample.
Discussion: SNP-arrays suggested genomic instability in approximately 30% of patients, often associated with gain-of-function variants in several oncogenes (NRAS, KRAS, BRAF) and loss-of-function variants in TP53, related to the risk of progression, survival, and resistance to therapy. Moreover, we detected variants in DNMT3A gene correlated with tumor progression. Furthermore, sWGS detected CNA and LOH with superior sensitivity compared to array and, with a specific bioinformatic pipeline, was able to identify balanced translocations, demonstrating sWGS is superior to FISH, which is not able to identify all CNA and LOH.
Conclusions: sWGS has proven to be a superior method for identifying biallelic events and rearrangements with heterogeneous breakpoints. WES and sWGS, to be used as a first diagnostic approach, can be helpful in addressing various aspects related to targeted therapy, therapy failure and relapse, providing crucial information on clinical decision-making.

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RARE DISEASES

Expanding the Phenotypic Spectrum of PPP2R5D-Related Neurodevelopmental Disorder

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The PPP2R5D gene is associated with a neurodevelopmental disorder, of which, to date, almost 100 cases have been described. The clinical phenotype is characterized by mild to severe intellectual disability, macrocephaly, hypotonia, epilepsy, nonspecific facial features, and, less commonly, early onset parkinsonism and congenital malformations. Nearly all reported causative variants in PPP2R5D are de novo missense variants and cluster in the acid loop domain (amino acid positions 197-253), a highly conserved region responsible for interactions with other PP2A subunits.

We describe 4 patients, including 3 unrelated subjects and 3 from one family, in whom we found 4 different PPP2R5D variants by means of massive parallel sequencing of a panel of genes responsible for syndromic macrocephaly. All variants cluster within the hotspot region, with 3 of them being de novo and already reported in scientific literature. Conversely, the familial variant NM_006245.4:c.598GA(p.Glu200Lys) had never been described and was identified in the apparently healthy mother of two boys with neurodevelopmental disorders and macrosomia. Notably, at the age of 15, the older brother exhibited a fine unilateral tremor of the upper limb.

Our findings provide further insights into the phenotypic spectrum of PPP2R5D-related neurodevelopmental disorder. Considering both the very mild phenotype displayed by some patients and the fact that we found the four variants in a cohort of 78 patients referred to us with macrocephaly and cognitive impairment, this condition may be more frequent than expected in the population, with significant clinical impact related to the suggested risk for early onset parkinsonism.

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RARE DISEASES

Utility of Optical Genome Mapping in the characterization of structural rearrangements in rare diseases: analysis of a multi-centre series of 66 cases

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Optical Genome Mapping (OGM) is a recently developed technique able of providing the same information as karyotype at a much higher resolution, marking a decisive turning point for cytogenetics. Working on high weight DNA molecules, labelled according to a uniquely recognizable pattern, it allows detecting and characterizing copy number and structural variants (CNVs and SVs), providing the missing piece of information between microarray and sequencing techniques.

The present multicentre study was promoted as part of the Cytogenomics, Prenatal and Reproductive Genetics Working Group (SIGU) activities and all samples were analysed at UOC Medical Genetics of OPBG Hospital. It aims to verify the reliability and the detection rate of OGM on a strictly selected cohort of 66 patients, enrolled according to specific criteria and classified as: A) Patients harbouring a karyotype-visible rearrangement or CNVs suspected to be associated with structural anomalies (n. 25). B) Patients presenting with a clinical diagnosis for a dominant condition tested negative at gene-specific analysis (n. 16). C) Patients in which sequencing analysis showed a single pathogenic variant in a recessive disease-causing gene (n. 19). D) Patients presenting with genetic condition usually referred to a small gene panel, tested negative at sequencing (n. 6).

OGM characterized all the 25 SVs included in group A. In 5/25, OGM allowed detecting the interruption of a dominant disease-causing gene, increasing the diagnostic yield by 20%. Moreover, in 4/11 cases (36.4%) with single/multiple CNVs it revealed an underlying causative structural event. In 3/16 (18.8%) patients of group B, OGM showed an SV involving the disease-gene sequence. These 3 patients presented with neurofibromatosis type 1, whose causative gene (NF1) appears therefore particularly prone to undergo

structural events. In 1/19 (5%) patient of group C a deletion was detected as a second hit in a recessive gene. No variants were detected in patients of group D.

OGM has proven to be a powerful tool to detect and characterize SVs, highly increasing the diagnostic yield. It confirms a non-negligible rate of CNVs also in “Mendelian phenotypes” and, interestingly, it allowed detecting recurrent rearrangements in these cases, expanding the molecular spectrum of specific diseases.

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RARE DISEASES

The EU-IHI Screen4Care project: two-tiers genetic newborn screening by an innovative customized TREAT panel and whole genome sequencing in rare diseases

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The Screen4Care is a research project funded by EU-IHI with the aim of shortening the path to diagnosis for rare diseases through genetic newborn screening (gNBS) and digital technologies. It is composed of 5 work packages dedicated to different activities and tasks. We have designed a gNBS pipeline we plan to apply on up to 25.000 neonates in Italy, Germany, France, and Greece, for early identification of rare diseases (RDs) using a NGS-based gene panel (TREAT panel).
We adopted stringent and specific criteria and scoring as treatability (mandatory criteria), mendelian inheritance, natural history knowledge, clinical utility, and amenability of NGS.
We selected by cut off-narrowed scores 245 genes, which are associated with the following phenomics: 106 metabolic, 33 blood and coagulation, 29 endocrine, 26 immunological, 25 neurological and neuromuscular, 9 renal, 6 mendelian syndromes, 4 cardiological, and 7 other phenotypes. Among them, 168 have autosomal recessive, 24 autosomal dominant, 20 X-linked, and 33 both AD and AR inheritance. In infants screened by TREAT panel but resulted negative and became symptomatic during the first year of life we offer WGS in a two-tier, post-NBS, enrolment approach. This will allow us to identify the 245 gene-related diseases and many other RDs that can be picked at the neonatal age by whole genome sequencing (WGS), thus allowing to know the frequency of early symptomatic RDs escaping the TREAT panel detection. The 24 European Reference Networks (ERNs) will be involved for the diagnostic work-up, treatment referral, and follow-up. S4C would propose and possibly translate its research output into the diverse European health systems for an equal EU gNBS approach.

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RARE DISEASES

DECIPHERING THE PHENOTYPIC SPECTRUM OF RATARS SYNDROME DUE TO SPEN HAPLOINSUFFICIENCY

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Radio-Tartaglia syndrome (RATARS; MIM#619312) is a rare neurodevelopmental disorder characterized by neurodevelopmental delay/intellectual disability, behavioral abnormalities and recognizable craniofacial dysmorphism. The phenotype is complicated by precocious puberty and obesity in females. Congenital heart defects, seizures and/or pyramidal signs are reported in a subset of individuals. The clinical features overlap those observed in patients with chromosome 1p36 deletion syndrome. The disorder has recently been related with SPEN haploinsufficiency in 34 individuals (PMID:33596411).

We used clinical data from 32 additional individuals with truncating SPEN variants to better define the phenotype of RATARS.

The clinical profile invariably included developmental delay/intellectual disability. Behavioural/psychiatric abnormalities (e.g., autistic features, anxiety, aggressive behavior and attention deficit disorder) were noted

in 81% of individuals. Othe major features were hypotonia (73%), brain and spine anomalies (64%), congenital heart defects (28%), high/narrow palate (32%) and facial dysmorphisms (i.e., broad forehead, frontal bossing, bitemporal narrowing, arched elongated eyebrows, synophrys, telecanthus, epicanthal folds, dysplastic ears, and broad nose with bulbous/prominent nasal tip). Precocious puberty and obesity/increased BMI were confirmed as common features in females.

Our data provide a deeper insight into the clinical and molecular variability of RATARS documenting the long-term complications and depicting the natural history of the disease in a novel large cohort of individuals carrying truncating SPEN variants.

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ETHICAL, LEGAL AND SOCIAL ISSUES ON HUMAN GENETICS

THE CURRENT STATE OF LATIN AMERICAN CONTRIBUTION TO PUBLICATIONS IN CLINICAL GENETICS: A BIBLIOMETRIC ANALYSIS

MD Paulo Victor Zattar Ribeiro[1], Dillan Cunha Amaral[2], Ana Luiza Machado Ribeiro Pimentel[3], Adriano Henrique Gomes Menezes[2], Luan Cavalcante Vilaça Lima[2], Lisandra Letícia Palaro[4], Gabriela Vera Rodriguez[4]

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Introduction/Background: Clinical genetics has an important role in providing a definitive diagnosis for many rare syndromes. Refereed clinical genetics journals are the reporting vehicle for influencing over genetics prevention, and disease prognosis and assisting the selection of the best options of treatment for patients. However, disparities in access to appropriate health care, including access to clinical genetic services, and health literacy among the Latin American population may have a significant impact on the burden of the amount of publication and impact in the field. Methods: This bibliometric analysis was conducted through the Scopus database (Elsevier) with journals present in the subject area of Genetics (clinical). The journals were ranked using the Cite Score metric. We cover all thematics areas of the "Clinical Genetics" category, all types of scientific publications in English and all countries from inception through February 20, 2024. The data was found using "Country/Territory" presented in each magazine from SCOPUS website and the analysis was made using Microsoft Excel and Numbers. Results: After excluding general medical journals, journals published in a language other than English, and titles unrelated to the clinical genetics field, the Scopus database was found to list 139 clinical genetics journals that have published 334.042 articles, from which 32 are open access by SJR, constituting the data set for this analysis. America Latina´s participation in the total was 12.615 articles, corresponding to 3.77%. The country with the highest contribution was Brazil, with 47.7% of the articles from Latin America amount, followed by Argentina, with 16.6%. When analyzing the 10 journals with the highest impact factor by the Scimago Journal Rank Indicator, the Latin America contribution is 0.37%. Conclusions: Medical genetics services have had a fragmentary and uneven development in Latin America, being mostly concentrated in wealthy, urban areas. This is also reflected by scientific contribution, which is still few when globally compared. In conclusion, the growth of human and medical genetics demands trained physicians and scientists in both basic and translational applications.

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RARE DISEASES

NEW CLINICAL FINDINGS IN SHASHI PENA SYNDROME DUE TO ASXL2 MUTATION: CASE-BASED REVIEW OF LITERATURE

MD Paulo Victor Zattar Ribeiro[1], Ester Silveira Ramos[1], Carolina Paes Torres[2], Bruno Bordest Lima[1], Adolfo Marcondes Neto[1], Lucca B. Palavani[3], Luan Cavalcante Vilaça Lima[4], Dillan Cunha Amaral[5]

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Shashi Pena syndrome (SHAPNS) is a rare and newly recognized neurodevelopmental disorder with unique phenotypic features, that was described for the first time in 2016. The syndrome is caused by truncating variants in the ASXL2 gene. We presented a 13-year-old girl who started a genetic investigation because of tall stature, intellectual delay and facial dysmorphisms. During her childhood she presented with development delay, speaking her first words at the age of three. Today she remains with mild intellectual disabilities. As important manifestations, she presented with two febrile convulsions, severe scoliosis, asymmetry of legs, cardiac findings such as non compacted myocardium, and aortic insufficiency. As neurologic image findings she presented with whitematter volume loss, she also presented with hepatomegaly in the abdominal ultrassom. As dysmorphic findings we could notice: epicanthal folds, hypertelorism, arched eyebrows, prominent glabella, and broad nasal tip, ptosis of eyelids, tall stature, separated teeth, high and narrow palate, positive wrist and thumb test. She has no history of genetic diseases in her family. She was submitted to a genome analysis, using a new sequence generation sequencing, and it was found a probable pathogenic loss of function variant c.2707CT p.(Gln903Ter) in heterozygous in the ASXL2 gene associated with SHAPNS. It´s known that SHAPNS common clinical features are neurological and neurodevelopmental disorders with some facial features, whereas differences in the clinical phenotypes for each individual could be found. As similar important characteristics previously reported in the individuals with this syndrome , our patient has: white matter volume loss, difficulties of feeding in the neonatal periodic, intellectual disability, classic facial and dysmorphic findings, speech delay, history of febrile convulsions, development delay, congenital heart disease, scoliosis. As never reported clinical features, she presented with: marfanoid clinical findings such as tall stature and positive wrist and thumb test, non compacted myocardium and hepatomegaly. It was never before reported individuals with SHAPNS that had marfanoid characteristics and non compacted myocardium, the previous patient all presented with short stature and others congenital abnormalities. Understanding the possible new clinical findings is important to recognize the syndrome more easily.

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PRECISION HEALTH

A PILOT STUDY OF WHOLE GENOME SEQUENCING BASED NEWBORN SCREENING IN TÜRKIYE

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Despite the advancements in whole genome sequencing (WGS) facilitating newborn screening and genetic research, such studies remain underrepresented in the Middle East, especially Türkiye. Our study used the GenRiskPro, an automated, standardised genomic analysis pipeline, to examine the genetic structure of Turkish newborns and identify the risk and loci for various inherited conditions. We conducted WGS on 626 health newborns and 15 newborns with clinical disease phenotypes in Türkiye. The variants corresponded to childhood-onset diseases, adult-onset diseases, and carrier status of hereditary conditions were detailedly analysed. Together with the detected genome-wide association study (GWAS) loci and paediatric pharmacogenomics (PGx) information, final reports are generated and returned to study participants through our developed digital clinic system. Our results revealed Metabolic disorders and neoplastic syndrome and cancer as the most prevalent early and adult-onset diseases, where Phelan-McDermid syndrome and Arrhythmogenic right ventricular dysplasia 11 emerged as the condition with the highest carrier frequency, respectively. Of note, among all the predicted high disruptive variants, Gene PEX5 (c.147+77_147+121del) was identified in more than 80% of the newborns. Additionally, PGx analysis indicated a need for careful dosage adjustment of tacrolimus and warfarin in this cohort, and it suggested avoidance of antidepressants. This pioneering study of a large-scale Turkish newborn cohort provides comprehensive data and insights into the genomic landscape of Turkish neonates. The GenRiskPro pipeline with the digital clinic system also enhances clinician-patient communication and enables effective data archival for ongoing neonatal medical care and future research. While highlighting the importance of population-specific genomic studies in improving neonatal and paediatric healthcare in Türkiye, our findings inform more in-depth studies of the Turkish neonatal genome.

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TRANSGENERATIONAL INHERITANCE / EPIGENETICS

STUDYING THE IMPACT OF CLIMATE CHANGE ON HUMAN DNA AT BASE PAIR RESOLUTION

Studying the impact of climate change on human DNA Emilia Volpe[1], Luca Corda[1], Elena Di Tommaso[1], Franca Pelliccia[1], Riccardo Ottalevi[2], Danilo Licastro[3], Andrea Guarracino[4], Mattia Capulli[2], Giulio Formenti[5], Evelyne Tassone[1], Simona Giunta[1]

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In recent years, rapid advancements in sequencing technologies and, in particular, the emergence of third-generation sequencing, have revolutionized genomics, rendering fields as de novo genome assembly more accessible.

Here, we propose a new concept based on the desire to create a completely matched reference to map and analyze omics-data in an isogenomic manner.

With this work, we are studying the impact of climate change on human DNA. This calls for the need of an unbiased reference, to acknowledge the true specific mutations induced by environmental agents, at a base pair resolution, like as Single Nucleotide Polymorphisms.

With this goal, we assembled the non-tumoral karyotypically stable hTERT RPE-1 diploid human cell line, which is one of the top three used in research laboratory worldwide.

To assemble this cell line, we used: PacBio High Fidelity, Oxford Nanopore Technology and Hi-C.

The final diploid genome was then used to evaluate alignment metrics and epigenetics analysis improvement when RPE-1 reads were mapped against their own genome instead of the non-matched one.

Diploid phased alignment of RPE-1 HiFi reads is equally distributed between each haplotype but secondary alignments and multimapped reads increased when aligned against the CHM13 genome.

We then tested the improvements of the mapped reads against the repetitive regions of the human genome, like the highly polymorphic centromeres.

When mapping to these regions, we observed a lower coverage or absence of mapped reads for the CHM13 alignment, while a complete uniform coverage for the RPE-1.

However, when their own genome was used as reference, two different batches of RPE-1 raw reads showed an increase in mapping quality and decrease in edit distance.

Once we established an overall improvement in alignment accuracy, RPE-1 reads, from Directed Methylation Long reads sequencing experiments, were analyzed to test differences in methylation profiles and CENP-A deposition using both RPE-1 genome and CHM13.

Using the non-matched reference genome, we observed a presence of homogeneous CpG methylation within centromeric αSat arrays, which contrasts their expected localized reduction. Furthermore, we did not observe CENP-A protein peaks corresponding to unmethylated centromeric regions. In addition, CENP-A shows a coverage decrease along all chromosomes.

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COMPUTATIONAL BIOLOGY AND AI

STRUCTURAL INSIGHTS INTO THE CENTROMERE: COMPARATIVE ANALYSIS OF SECONDARY STRUCTURE AND THEIR STABILITIES

Structural Insights into the Centromere Secondary Sai Swaroop Chittoor[1], Simona Giunta[1]

Dpt. Biology & Biotechnology Charles Darwin, University of Rome "La Sapienza"., Giunta Laboratory of Genome Evolution, Italy

The centromere is an essential part of the chromosome given its role in orchestrating equal distribution of the genetic material during cell division. The core centromere comprises a domain termed the “active region”, composed of repetitive α-satellite DNA organized in Higher Order Repeat (HORs) blocks that are reiterated in tandem in a near-identical fashion throughout the centromere. In the active HOR, a hypomethylated region named the “CDR`` (Centromeric Dip Region) has been recently identified which correlates with a higher density of the histone H3 variant CENP-A (centromeric protein-A) containing-nucleosomes, likely marking the functional loci for kinetochore assembly in mitosis. The active HOR region is flanked by the pericentromere on both sides where it progressively loses its sequence identity. The pericentromeric region contains the “inactive HORs`` that transition into the “divergent region” containing regions of diverged αSat HORs whose periodicity is eroded along with highly divergent αSat monomers which lack a HOR structure. The pericentromere is a genetically and epigenetically distinct region that harbors heterochromatic marks, divergent DNA repeats, transposons, and other genetic elements. Various types of satellite DNA with distinct characteristics are present in the pericentromere. Interspersed between the satellites as well as flanking the pericentromere on both the chromosome arms is the “centric transition” region which hosts segmental duplications, lncRNAs as well as genes. In this work, we evaluate the intrinsic properties of linear DNA sequences of the centromere and pericentromere of the CHM13 human reference assembly. Using DNA secondary structure prediction software, we obtained the secondary structures predicted to form by the repetitive genomic loci within the centromere and compared their stabilities with those of the pericentromere, rDNA and unique gene-coding regions. We found that the stability and integrity of the secondary structures decreases as we move closer to the core centromeric regions compared to the flanking pericentromere or towards the chromosome arms. Our data confirms the notion that centromeric repeats have intrinsic self-hybridizing properties that can generate complex high order non-B DNA structures when DNA presents in a linear form, including during DNA-based transactions such as replication, transcription, and repair.

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GENETICS OF COMPLEX DISEASES

Calibration of Polygenic Risk Scores in African Americans using Estimated Genetic Ancestry

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Background

Polygenic risk scores (PRS) are an emerging clinical risk factor for common disease. The PRS distribution is a function of allele frequencies that can vary considerably between different contributing continental ancestry populations. We have identified three different PRS for coronary heart disease (CHD); all three scores have means and variances that are strongly associated with proportion of estimated African ancestry. In this study, we investigated how calibrating PRSs, accounting for proportion of African ancestry in each individual, impacts PRSs performance in predicting coronary heart disease (CHD) among African Americans (AAs) participating in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study.

Methods

Analyses included 6,634 AA, including 919 with incident CHD, and three PRSs for CHD (pgscatalog.org: PGP000466, PGP000232, PGP000005). For each individual, we calibrated their PRS score by regressing out the predicted value of the PRS as a function of their estimated African ancestry and dividing by the standard deviation specific to their estimated African ancestry decile. Individuals were classified as “at-risk” if their PRS was in the upper 10% of the PRS distribution in the entire AA group. We used logistic regression models to test for the effect of pre- and post-calibrated PRSs CHD, while adjusting for sex, age, and estimated African genetic ancestry. Model performance for the PRSs was evaluated using the change in area under the curve (AUC).

Results

We observed that CHD PRSs functions in AAs are significantly skewed by proportion of African ancestry. CHD incidence is not associated with proportion of African ancestry in REGARDS AAs. PRSs either under- or over-estimated risk in individuals with increased estimated European ancestry. For PGP000005, all AA individuals with 60% estimated African ancestry were systematically excluded from the “at-risk” classification. After standardization, PGP000005 performance for this subset of individuals increased significantly (AUC=0.71, 95%CI[0.59-0.87]). Nonetheless, the correction by ancestry did not significantly improve the overall performance of the PRSs. The PRSs have relatively low overall predictive value for CHD (AUC~0.62) in AAs. Our results show there are small gains in CHD prediction for the relatively small subset of AA individuals with higher European ancestry.

COMPUTATIONAL BIOLOGY AND AI

Amplicon-based CNV precision detection: the BRCA1 and BRCA2 approach

Dr Stefano Casali[1], Francesco Zapelloni[1], Valentina Favalli[1], Gianluca Vozza[1]
4bases Italia srl, Italy

Introduction

The issue of correctly evaluate copy number variations (CNVs) in amplicon-based sequencing is well known in NGS, for small panels. The correct balance between sensitivity and specificity, must consider the need for quick but robust reporting.

This study describes three separate datasets generated using the BRCA panel kit from 4bases, with a primary goal of detecting germline CNVs in these samples. Each dataset comprised known CNVs, serving as a benchmark for the validity of our detection approach.

Methodology

We employed a consensus-based method, incorporating three CNV detection tools. This triangulated approach enabled a thorough evaluation of CNVs, providing a score for each sample indicative of the likelihood of either deletion or duplication.

The scoring mechanism was clear-cut: a zero score implied no detection of CNVs by any tools; a score of 0.3 indicated detection by a single tool; a score of 0.7 suggested detection by two tools; and a perfect score of 1 meant that all three tools detected the CNV. A CNV was considered present if it achieved a score of 0.7 or higher. In contrast, a score of 0.3, indicating minimal evidence of a CNV, was deemed a negative result.

This methodological framework aims to enhance the detection accuracy of CNVs in the context of genetic testing using the BRCA panel kit, offering insights into the efficiency of the employed tools.

Results

The use of diverse CNV detection tools, coupled with a systematic scoring mechanism, enabled a comprehensive analysis of BRCA germline CNVs. With observed sensitivity and specificity rates consistently above 0.99, this method shows promising accuracy in identifying CNVs while minimizing false positives.

Conclusion

This study highlights the robustness and precision of our consensus-based approach in detecting CNVs using the BRCA panel kit.

It is important to note that the effectiveness of this analysis is influenced by coverage content, sample and run quality.

Future efforts will consider effectiveness in broader genetic testing scenarios.While further refinement is ongoing, our approach is proving to be a valuable tool in genetic diagnostics.

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CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

Unveiling a novel physiological LRIG2 isoform: implications for Urofacial syndrome and motor neuron differentiation

Ornella Rondinone[1], Matteo Uggeri[2], Alessia Mauri[3], Giada Moresco[1], Jole Costanza[4], Carlo Santaniello[3], Patrizia Colapietro[5], Monica Nizzardo[6], Giovanni Marfia[7,8], Laura Guarnaccia[7], Stefania Navone[7], Gianantonio Manzoni[9], Federico Grilli[10], Donatella Milani[10], Pasqualina D'Ursi[2], Monica Rosa Miozzo[1,11], Laura Fontana[1,11]

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Urofacial Syndrome 1 (UFS1) is a genetic disorder characterized by congenital urinary dysfunction caused by mutations in the LRIG2 gene, which plays a crucial role in embryonic neural migration and cancer cell proliferation. Despite its known functions, the involvement of LRIG2 in bladder innervation and UFS pathogenesis remains elusive.

By WES, we found a novel homozygous splicing mutation (c.1478-2AG) in LRIG2 gene in two siblings with suspected UFS, leading to an isoform lacking exon 13 (LRIG2-del-13) and, thus, the first Ig-like domain in the encoded protein. The effect of the mutation was confirmed by LRIG2 mRNA expression analysis in the probands, who showed the expression of the LRIG2-del-13 only, while their heterozygous parents expressed both the LRIG2 wild-type and LRIG2-del-13, as expected.

We assessed the expression of wild-type and novel isoform in various tissues and differentiated motor neurons from induced pluripotent stem cells (iPSCs). The LRIG2-del-13 isoform, missing the first Ig-like domain, was found to be physiologically expressed across different tissues, albeit at lower levels than its full-length counterpart. Notably, its expression in iPSCs and the subsequent silencing in differentiated motor neurons suggests a role in neuronal differentiation, and particularly stemness maintenance in neuron progenitors. In silico protein modeling analyses revealed that the LRIG2-del-13 isoform is likely to inhibit the formation of functional homodimers, indicating its altered activity or mechanisms compared to the full-length protein. This hypothesis is supported by the analysis of other LRIG2 variants affecting the Ig1 domain`s structure.

In conclusion, this specific splicing mutation gave us the opportunity to identify and characterize a new LRIG2 isoform, shedding light on its potential roles in UFS pathogenesis and neural differentiation processes. This insight opens new research avenues into the mechanisms underlying bladder innervation and motor neuropathy in UFS.

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RARE DISEASES

The value of SNP-arrays analysis in pregnancy losses: a retrospective study

Stefania Cappellani[1], Adamo Pio d'Adamo[1,2], Beatrice Spedicati[1,2], Elisa Paccagnella[1], Stefania Zampieri[1], Diego Favretto[1], Giovanni Turchetto[1], Davide De Santo[3], Gianpaolo Maso[3], Sheila Ulivi[1], Giorgia Girotto[1,2], Paolo Gasparini[1,2]

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Introduction: Spontaneous terminations of pregnancy (SToP) are pregnancy losses in any gestational age after a clinically confirmation of pregnancy, and may be due to infections, placental pathology, umbilical cord complications, maternal conditions, congenital malformations and chromosomal abnormalities. Cytogenetic and cytogenomic analysis of either products of conception or spontaneously lost fetuses are recommended as well as histological exanimation of both placenta and fetal autopsy tissue. Despite an extensive work-up, a substantial proportion of SToP, ranging from 25 to 60%, remains unexplained.

Aim of the study: To investigate the aetiology and evaluate the clinical utility and the detection rate of SNP-arrays analysis in a cohort of SToP.

Materials and methods: Clinical and gestational data of SToP from any trimester were retrospectively collected. A comprehensive etiological investigation, including maternal and prenatal history, feto/placenta-pathological examination and in-trio SNP-array analysis, was conducted.

Results: 64 cases of SToP were selected, including 29 miscarriages in the first trimester, 21 late pregnancy loss in the second trimester and 14 in-utero fetal death in the third trimester. Maternal median age was 35 years (23-47). DNA was extracted from fetal tissue (32), placenta (3) or fetal skin (29). SNP-arrays allowed to solve 13 cases (20%). Among them, 12 chromosomal abnormalities were identified in StoP occurred in first trimester miscarriages: three triploidies, seven autosomal trisomies (in particular trisomy 8, 11, 13, 21 e 22), one monosomy 21, and one concomitant presence of trisomy 21 and 22. A duplication of approximately 2.5 Mb in 22q11.21, comparable to the 22q11 duplication syndrome, was identified in the thirteenth case concerning an in-utero fetal death. However, this result did not completely explain the SToP.

Conclusions: In our cohort, the presence of chromosomal abnormalities significantly contributed to explain only the cases of SToP occurred in the first trimester, confirming the results previously observed in literature.

SNP-array ruled out genomic abnormalities in StoP during second and third trimester and, therefore, further studies, such as Whole Genome Sequencing, will be required to better understand the mechanisms leading to late pregnancy losses.

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RARE DISEASES

The impact of Whole Exome Sequencing for the molecular diagnosis of fetal malformations: the experience of a referral Italian hospital

Professor Stefania Zampieri[1], Agnese Feresin[2], Anna Morgan[1], Elisa Paccagnella[1], Daniela Mazzà[1], Beatrice Spedicati[1,2], Tamara Stampalija[2,3], Maurizio Pinamonti[4], Rossana Bussani[2,4], Flora Murru[5], Giorgia Girotto[1,2]

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Introduction: Approximately 3% of pregnancies are complicated by fetal structural anomalies, ranging from isolated minor anomalies to severe multisystem disorders, which are usually identified during the second trimester. Whole Exome Sequencing (WES) is indicated as a second-tier test in case of a major malformation or multiple anomalies after a normal karyotype or chromosomal microarray analysis.

Aim of the study: This study aims to analyze a deeply characterized cohort of fetuses with structural anomalies and to highlight the value of WES as a fundamental diagnostic tool in the prenatal setting.

Materials and methods: A cohort of 68 fetal cases, from 62 families, characterized by structural anomalies and without aneuploidies or pathogenic copy number variants and a fulfilled post-mortem evaluation, were selected for WES analysis. For all fetuses, detailed phenotypic information (i.e., dysmorphology examination, histology, and imaging) and fetal tissue were available. The obtained data were discussed in multidisciplinary meetings.

Results: Data analysis led to the identification of “likely pathogenic variants” or suggestive “variants of unknown significance” in genes with an already established genotype/phenotype association in 24 cases (39.7%). Interestingly, it was also possible to detect already-known pathogenic variants previously reported only in postnatal conditions, expanding the knowledge of clinical symptoms in the prenatal setting. As an example, a pathogenic variant in the EBP gene (c.364GA, p.(Glu122Lys)) was identified in a female fetus with severe skeletal dysplasia. This variant was previously described in an adult woman affected by Conradi–Hünermann–Happle syndrome, a rare X-linked dominant condition.

Conclusions: This study highlights the value of WES in the diagnosis of fetal malformations, allowing to perform couples counseling and define specific recurrence risks, and providing clinical relevant information regarding novel fetal genotype-phenotype associations. Further investigations (e.g. Short/Long reads Whole Genome Sequencing) will be carried out for unsolved cases.

POSTER SESSION 2
APRIL 09
FROM 10:15 TO 11:00