ORAL PRESENTATIONS

#500 - Genetic Therapy for Hearing Loss and Vestibular Dysfunction

Requested topic(s): GENE THERAPY

Requested presentation type: Poster presentation

Abstract Details

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Abstract
The auditory and vestibular systems, respectively, define the ability to hear sounds and to maintain balance. Both systems depend on many synchronized processes, including coding genes and regulatory elements. More than 150 genes have been identified that play a role in the normal function of the human auditory system, and a significant effort has been made to understand the mechanisms of hearing loss, as it affects approximately 6-8% of the world's population. Although more than half of cases are due to genetic variants, most treatments today include amplifying hearing aids and cochlear implants. Therefore, other therapeutic strategies, such as biological treatment, are essential for curing genetic hearing loss, and the potential for rescue needs to be examined. Here, we aim to restore both vestibular and auditory functions in the Clic5 mutant mice, a gene associated with hearing loss and vestibular dysfunction. We created two synthetic AAV2/9-PHP.B vectors to evaluate therapeutic efficacy: self-complementary AAV (scAAV) and single-stranded AAV (ssAAV). Subsequent to their injection into the utricle of Clic5c.680T>C mice at P0, we assessed both hearing through auditory brainstem response (ABR) and distortion product optoacoustic-emission (DPOAE) tests. Additionally, we examined vestibular function using open field, rotarod, and swimming tests.

We observed vestibular function recovery and improved hearing, with some cases showing auditory brainstem response thresholds close to those of wild-type mice for each AAV vector. Hopefully, this research will contribute to advancing translational research to treat hearing and balance disorders in humans in the future.

Research supported by the U.S.-Israel Binational Science Foundation.

#465 - GENETIC FACTORS IN TYPE 1 DIABETES AMONG THE QATARI POPULATION: IDENTIFICATION OF HLA GENOTYPES ASSOCIATED WITH ENHANCED OR REDUCED RISK OF DISEASE ONSET.

Requested topic(s): GENETICS OF COMPLEX DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Background: Recent epidemiological data shows that Qatar has the 4th uppermost incidence of typel diabetes (T1D) globally alongside Finland and Sweden. T1D is linked with considerable heritable risk, particularly from human leukocyte antigen (HLA) alleles. Population of Qatar is distinguished by unique genetic subgroups, high levels of consanguinity, and admixture from early migration. This implies that our population may have a very distinctive HLA landscape, which is relevant for clinical use but has not yet been investigated.

Aim: Fine mapping of the HLA alleles and DR-DQ haplotypes for 15k Qatar Biobank subjects and an in-house recruited T1D cohort. Assessment of the risk and protective HLA alleles and haplotypes that exist in our population.

Methods: HLA type inference was performed using multiple independent typing methods with high accuracy on the WGS data. Assessment of DR-DQ haplotypes was performed using maximum likelihood estimates of haplotype probabilities, and association analysis was conducted to understand the susceptibility of alleles and haplotypes to T1D.

Results: We found a high diversity of rare alleles among class II HLA genes in our population. Multiple alleles from genes DRB1, DQA1 and DQB1 which are known to segregate with T1D predisposition showed a significant association with T1D. The heterozygous genotype DR3/DR4 conferred the highest risk (OR=6.25) compared to the homozygous genotypes DR3/DR3 (OR=5.84) or DR4/DR4 (OR=3.36). Our results suggest a greater genetic susceptibility for T1D in the general population through known and novel haplotypes and thus provides an opportunity for the discovery of these novel associations.

Conclusion: We present a genetically distinct landscape of the HLA locus for a consanguineous population with enrichment for several potentially protective and risk alleles for the onset of T1D.

#271 - DISSECTING THE BIOMEDICAL AND GENETIC STRUCTURE OF A SOUTHERN ITALIAN COHORT: THE MOLI-SANI STUDY

Requested topic(s): GENETICS OF COMPLEX DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract

The reservoir of human genetic diversity in Southern Europe has been historically undersampled owing to a paucity of large-scale genomic initiatives. In Italy, complex demographic events and a unique geography contributed to high levels of genetic heterogeneity. To characterise factors affecting the architecture of biomedical traits and diseases, we carried out the largest to date genomic survey in Italy using the Moli-sani study, a prospective cohort of approximately 25,000 individuals (age ≥ 35, 49.3% men), recruited from city hall registries in the Molise region.

We newly profiled genome-wide SNPs and used them to (i) address genetic structure vis-a-vis other Italian and European populations; (ii) describe genetic contributions to over fifty biomedical traits relevant for chronic-degenerative diseases; (iii) assess the portability of polygenic risk scores (PRSs) estimated from other European populations in South Italy.

While confirming a broad genetic overlap with Northern European populations, our GWASs have identified 73 new SNPs and 10 new loci associated with 7 biomedical traits. As an example, we have identified different variants near the HBB gene showing greater allelic frequencies and per-allele effect size in Moli-sani than UK Biobank. Among these, we observe rs11549407 and rs35004220, known to be involved in beta-thalassemia disorders in South Europe and likely reflecting past historical events. Overall, we estimate that PRSs, developed from European summary statistics, explain ~20% less variance compared to estimates in Northern populations.

The genetic profiling of the Moli-sani cohort, coupled with its large size, depth of phenotyping, and prospective design, provides a unique resource to study genetic contributions to common diseases and traits, and the extent to which these are modulated in the context of South Italy's unique history and environments.

#341 - CHARACTERIZATION AND AGING ASSESSMENT OF HUMAN INDUCED PLURIPOTENT AND MESENCHYMAL STEM CELLS IN MDPL SYNDROME

Requested topic(s): RARE DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract

#453 - A systematic approach for thousand severe unsolved pediatric conditions: results from the Telethon Undiagnosed Disease Program

Requested topic(s): RARE DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract
Many sporadic and serious childhood diseases remain undiagnosed despite extensive medical and genetic testing. Diagnosis is critical for prognosis, specific and timely treatment, family planning, and understanding the pathomechanisms of disease.

In Italy, the Telethon Foundation charity created the Telethon Undiagnosed Diseases Program (TUDP) to provide a systematic approach to improve the diagnostic yield and provide an overview of the underlying genetic mechanisms.

All applications (1,232) were submitted together with electronic phenotyping from approximately 60 clinical geneticists from 22 pediatric genetics and clinical centers or via an online platform. Procedures were standardized across centers through regular online meetings to achieve more accurate and reproducible phenotyping. Eligibility criteria were based on severity, complexity, and negative results of complete genetic analysis. Genetic testing was performed at least in trios, and the case reanalysis and matching process was repeated periodically for all still-negative cases. The TUDP has studied 1,063 families selected from 1,232 applications. 897 clinical genetic reports have been provided to date. A definitive diagnosis has been made in 50% of cases, with mutations identified in more than 200 different genes. In addition, about 10% of cases have suspect variants that are used in the matching process to find second cases. The general overview indicates that the vast majority (71%) of the causative variants were de novo, either with autosomal dominant (68%) or X-linked (3%) alleles. Recessive forms (either autosomal or X-linked) explained the remaining 29% of cases, with homozygous mutations identified in only 10% of cases. The high percentage of de novo mutations is similar to other undiagnosed programs in developed countries and may reflect the general postponement of parenthood. This confirms the need for parallel trio testing.

We conclude that a systematic approach can solve about 50% of missed diagnoses due to heterogeneity of genetic causes, while the remaining cases may be due to unique and therefore elusive mechanisms that require extended matching. The multicenter TUDP model is a cost-effective solution that should be transferred to clinical settings to avoid diagnostic delays or misdiagnosis in children.

#346 - Optimizing Structural Variant Calling: towards a robust and reliable detection from Whole Genome Sequencing (WGS)

Requested topic(s): COMPUTATIONAL BIOLOGY AND AI

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Introduction: Structural Variants (SV) are the most common variants occurring in the human genome, shaping heritable differences in gene expression and contributing to the onset of rare diseases. In the last years, WGS has helped unravel SV¨s complex nature. However, SV detection from WGS can result in a high number of false calls that can undermine the detection of a causal SV. Our study aims to evaluate the best up-to-date methodologies in SV discovery and filtering to develop a tailored analysis pipeline to be implemented in the clinical setting.

Methods: Deletion variant calling and filtering was performed using DRAGEN 4.0 (Dv0) and 4.2 (Dv2) alone or in combination with Duphold or SV-channel on the reference sample HG002. Benchmarking was carried out using Witty.er, calculating F1 score (F1), Precision (P), and Recall (R). Moreover, we evaluated performances in low-complexity regions (LCR) aligning data to a pangenome or hg38. Finally, to evaluate the validity of our approach, we tested the best performing methodology on three non-syndromic hereditary hearing loss trios negative to a) SNPs-array, b) Whole Exome Sequencing and c) WGS (focused on SNVs/ small INDELs) to possibly identify undetected disease-causing SV.

Results: Dv2 obtained the best performances (F1: 79%, P: 94%, R: 68%) across all deletions. However, Dv0 showed the best results in the detection of deletions ranging from 1000-5000 (F1: 93%, P: 97%, R: 90%) and 5000-10000 bp (F1: 95%, Precision: 97%, Recall: 93%). Moreover, analysing deletion variant calling in LCR, the use of the pangenome provided better results (F1: 81%, P: 90%, R:73%) compared to hg38 (F1:72%, P:96%, R: 58%). Finally, using our approach, we were able to solve one of our testing trios, detecting a novel 69 Kb duplication within *ATP2B2* shared by the proband and her affected father, thus providing for both a definitive diagnosis.

Conclusions: This study allowed us to solve one out of three tested cases using up-to-date methodologies for SV calling and filtering. In the future, we will evaluate other algorithms such as *dysgu* and *cue* in order to build a robust framework to be implemented in the clinical practice.

#495 - GENOMIC APPLICATIONS TO GLOBAL HEALTH: EXAMPLES FROM TUBERCULOSIS AND HEPATITIS B

Requested topic(s): GENETICS OF INFECTIOUS DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Despite tremendous overall progress in advancing global health, WHO's goals for diagnosing and treating tuberculosis have fallen well short of expectations. Currently the most significant cause of infectious disease mortality globally, more than 10 million people are sickened each year resulting in nearly 1.5 million premature deaths each year. Diagnosis of latent infection and patients at risk of developing active disease are major hurdles in improving outcomes. I will discuss recent advances from the field of genomics and human genetics, especially for the diagnosis of infectious disease using gene expression and other techniques.

HepatitisB (HBV) infects >250 million people. Some 5%-10% of exposed adults develop a chronic infection, causing liver damage, cirrhosis, or cancer in 15%-25% of cases. Vaccination has reduced infections, but there is no cure. We identify genetic variation impacting vaccine response, and the ability to spontaneously clear HBV in a cohort of Chinese individuals living in Taiwan.

The TaiwanBiobank comprises 120,552 adults tested serologically for HBsAg, anti-HBs, anti-HBc, and anti-HBe. Previously vaccinated, chronically, and formerly infected individuals were identified as shown (Table). Genotyping was performed with a custom array containing 591,048 SNPs.

We performed an unadjusted GWAS analysis on an early release of 2,000 chronically and 2,000 previously infected individuals. Highly associated SNPs ($p<5 \times 10^{-16}$) fall within a 36kb region, encompassing HLA-DPB1 and HLA-DPA1. There was no inflation of p-values due to population stratification. We have observed similar ClassII associations with vaccine response.
Shared HLA associations suggest those least protected by vaccination may be most at risk of chronic infection, a significant clinical concern. The inability to respond to vaccine and the risk of chronic infection are both characterized by absence of HBs antibodies. This in turn suggests that HLA ClassII protein sequences vary in their ability to bind and present s-antigen to B-cells, resulting in variable antibody responses. Future studies intend to directly examine binding affinities for s-antigen with HLA alleles identified here.

#344 - PEPTIDE-DESIGNED STRATEGIES TO COUNTERACT THE EVOLUTION OF SARS-COV-2 VARIANTS

Requested topic(s): GENETICS OF INFECTIOUS DISEASES

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Full eradication of COVID-19 is not on the horizon due to the emergence of new variants of concern and their vigilant monitoring is essential to enable the implementation as early as possible of necessary countermeasures. The inhibition of the interaction between the receptor-binding domain (RBD) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its receptors it routinely binds to infect human cells is an intriguing therapeutic approach to prevent the virus from entering human cells. Among other modalities developed for this purpose, peptides surely offer unique advantages, including ease of synthesis, serum stability, low immunogenicity, and low production costs. Moreover, their activity can be improved by changing amino acid sequence for increasing their affinities to the respective partner, as often determined by computational modelling. Here, by using a 3D mapping approach and docking simulations, we rationally designed a potent new inhibitor, based on the sequence of a previously identified peptide that has been designed on dipeptidyl peptidase 4 (DPP4) sequence, a ubiquitous membrane protein known to bind the RBD domain of the virus. This novel peptide results to be capable of targeting the latest tested variants, as revealed by in vitro testing in human Calu-3 cells. Specifically, the results obtained by the luciferase assay support the efficacy of the DPP4 derived peptide in reducing the VSV* DG-Fluc pseudovirus Omicron infection capacity up to 14% in a statistical manner, as also validated by a decrease in proinflammatory chemokine and cytokine levels. Surface plasmon resonance also confirmed the binding affinity of DPP4 derived peptide with the variant of concern occurring. On the base of these results, this peptide represents a promising candidate for the development of prophylactic or therapeutic treatments for SARS-CoV-2. These data demonstrate that the peptide-based technology represents a strategy able to dynamically and promptly counteract any viral infection, as it is possible to adapt weapons to fight it properly.

#406 - BREAST CANCER RISK POLYGENIC SCORE OPTIMISATION THROUGH A NOVEL SNP SELECTION ALGORITHM

Requested topic(s): CANCER GENOMICS

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Background/Objectives: Many Polygenic Scores (PGS) have been described for Breast Cancer (BC), differing by number and selection of SNPs. This variability leads to a wide range of estimated PGS in the same individual and therefore to inconsistent risk stratification. The aim of this study is to optimise the computation of BC risk scores by the selection of the variants involved.

Methods: Starting from the 100 PGSs available for BC on the PGS Catalog, we aligned all their variants (more than 7,000,000) to obtain the most represented SNPs associated to disease risk. The SNPs selection approach was based on the association with Breast Cancer and on the 100% concordance between the different PGSs. Weight normalization operations were performed by calculating Z-scores and incorporating the effect weights of each SNP to create a new PGS. Different types of approaches were used to calculate the new effects mean, sum and combined approach to give more importance to the weight of each variant.

Results: The newly created PGS has 4,606 SNPs. We tested this PGS on Genomic England, UK Biobank, FinnGen and HUNT data. In all the data sets tested the combined approach to estimate the effect weights shows the best performance, distributions between BC patients and controls were significantly different (Kolmogorov–Smirnov test p-value<0.0001). Across cohorts, women in the highest 1% of the score had almost three-fold increased risk of develop BC compared with women in the middle quintile. Lifetime risk of BC for women in the lowest and highest quintiles of the PRS was less than 10% and more than 20%, respectively.

Conclusion: These finding and optimization approach strengthen the standardisation of PGS that could help clinicians to better stratify patients risk of developing BC and may aid decision makers in formulating more reasonable and effective preventive health policies.

Grants: INTERVENE, HORIZON Project 2020 n.101016775

#327 - Combined measurements of chromatin and splicing in thousands of individual cells reveals convergent and divergent patterns between both modalities in health and disease.

Requested topic(s): SINGLE-CELL GENOMICS / SPATIAL GENOMICS

Requested presentation type: Oral presentation

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Abstract

Complex tissue, including the brain, often includes highly divergent cell types and these cell types employ distinct isoforms for many genes. To untangle the distinct cell-type specific isoform profiles of the brain, we developed the first single-cell long-read technology for >>1,000 cells for fresh tissues (ScISOr-Seq[1]) and frozen tissues (SnISOr-Seq[2]) and the spatial-resolution SI-ISO-Seq[3].

1. Gupta et al, Nature Biotechnology, 2018

2. Hardwick et al, Nature Biotechnology, 2022

3. Joglekar et al, Nature Communications, 2021

#255 - DONOR-DERIVED CELL FREE DNA IS ASSOCIATED WITH ANTIBODY-MEDIATED REJECTION IN PATIENTS WITH HEART TRANSPLANTATION

Requested topic(s): PRECISION HEALTH

Requested presentation type: Oral presentation

Abstract Details

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Abstract
Lifelong noninvasive rejection monitoring in heart transplant (HT) patients is a critical therapeutic need still inadequately provided in adults and even more for children.1

The historical and still current gold standard for assessment of cardiac allograft acute cellular and antibody-mediated rejection (ACR and AMR) is the endomyocardial biopsy (EMB).2

Recent developments in new sequencing technologies has allowed the concept of liquid biopsy to evolve, with the development of ad hoc genetic analysis, including the analysis of cell-free DNA (cfDNA) in the context of organ transplantation.3

The aim of the study is to assess the relationship between donor-derived cfDNA (dd-cfDNA) levels, ACR and AMR in a European real-life cohort of HT recipients.

dd-cfDNA was extracted from plasma derived from blood EDTA-tubes among the patients who received a protocol or for-cause EMB. dd-cfDNA was measured and sequenced using MiSeq Illumina platform, according to the AlloSeq cfDNA protocol (CareDx, San Francisco, CA) which evaluated 202 genomic polymorphisms of dd-cfDNA, compared to recipient patient as an index of rejection.

dd-cfDNA assessment was performed in 66 recipients. Median dd-cfDNA levels were significantly higher during the first month after HT (0.33% [IQR 0.19 to 0.47] in the first 30 days vs. 0.17% [IQR 0.12 to 0.23] after 30 days from HT). AMR was detected in 19 (28.8%) patients, in whom median dd-cfDNA levels were 0.23% (IQR 0.16 to 0.38%). Starting from 30 days after HT, dd-cfDNA levels were higher in recipients with AMR (p=0.04) as compared to those without AMR.

In conclusion, in a European real-life cohort of HT recipients, starting from 30 days after HT, AMR but not ACR was associated with higher dd-cfDNA levels. Meanwhile, we started to perform the analysis on pediatric HT patients to evaluate whether the dd-cfDNA percentage can be used as in adult cases as a proxy of AMR.

1. North et al., PloS One. 2022 Jan. 10.1371/journal.pone.0227385

2. Coutance et al., Biomolecules. 2022 Aug. 10.3390/biom12081135

3. Palmieri et al., JVS-Vasc Sci. 2020 Sep. 10.1016/j.jvssci.2020.08.002

#385 - EXTENDED CARRIER SCREENING IN CLINICAL PRACTICE

Requested topic(s): REPRODUCTIVE GENETICS

Requested presentation type: Oral presentation

Abstract Details

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Abstract

The GENNET CarrierTest is an extended carrier screening test based on a custom-designed NGS panel. It includes analysis of 65 genes with autosomal recessive inheritance and 7 X-linked genes.

We have examined in total 38088 individuals (18451 males and 19636 females), mainly couples with reproductive disorders and gamete donors. At least one mutation in these genes was found in 38.4% (1 mutation in 29.8%, 2 mutations in 7.26%, 3 mutations in 1.21%, 4 mutations in 0.12%, 5 mutations in 0,013%, 6 mutations in 0.003 %) of examined individuals. The genes with highest carrier frequencies are: HFE (6.95 %), CFTR (4.53%), GJB2 (3.79%), CYP21A2 (3.52%), DHCR7 (2.83%), SMN1 (2.66%), PAH (2.65%), SERPINA1 (2.60%), ATP7B (1.38%), PMM2 (1.21%), ACADM (1.12%).

Extended carrier screening should be available to all couples, even those without family history of inherited diseases. It is appropriate before treatment using assisted reproductive technologies. It allows detecting a disease risk before a first case in the family. The available options for couples at risk consist of preimplantation genetic testing of a monogenic disease (PGT-M), prenatal diagnosis, early postnatal diagnosis and treatment, as well as genetic matching for safe use of gamete donation.

#391 - GENETIC TESTING FOR MONOGENIC ETIOLOGY OF MALE INFERTILITY CONTRIBUTES TO THE CLINICAL DIAGNOSIS OF MEN WITH SEVERE IDIOPATHIC MALE INFERTILITY

Requested topic(s): REPRODUCTIVE GENETICS

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Background/Objectives: Infertility affects about 5% of adult human males. Despite the current diagnostic methods (karyotyping and Y chromosome microdeletion testing), around 70 % of men remain undiagnosed. In recent years, many genes have been proposed to be associated with this condition; however, testing of monogenic forms has not yet been clinically implemented in the diagnosis of severe forms of idiopathic male infertility, as the diagnostic utility has not been established yet. Aiming to develop a diagnostic test and identify the frequency of monogenic forms in a population of men with severe idiopathic male infertility, we defined a panel of 21 genes with sufficient evidence for the involvement with severe male infertility based on ClinGen curation protocol and performed whole exome sequencing analysis of men with significantly impaired spermatogenesis. Methods: Whole exome sequencing analysis was performed on 200 infertile men. DNA was prepared based on the Twist CORE exome protocol and sequenced on Illumina NovaSeq 6000 platform. All interesting variants were classified using the ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. Results: We identified monogenic disease-causing variants in four infertile men. Pathogenic/likely pathogenic variants in STAG3 (c.2776C>T, p.Arg926*; c.2817delG, p.Leu940fs), MSH4 (c.1392delG, p.Ile465fs; c.2261C>T, p.Ser754Leu), TEX15 (c.6848_6849delGA, p.Arg2283fs; c.6271dupA, p.Arg2091fs) and TEX14 (c.1021C>T, p.Arg341*) genes were found.Conclusion: In our study, we identified monogenic causes in 2 % of men with severe male infertility using a rigorously curated clinical gene panel. Our findings advocate for integrating of genetic testing for monogenic etiology into the diagnostic assessment owing to its clinical utility.

#235 - A NOVEL DEEP MUTATIONAL SCANNING APPROACH TO DISSECT AT SINGLE CELL LEVEL THE MOLECULAR BASES OF GENETIC DISEASES

Requested topic(s): CHALLENGES IN ASSESSING PATHOGENICITY OF VARIANTS

Requested presentation type: Oral presentation

Abstract Details

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Abstract

The improvement of sequencing technologies is quickly transforming research around rare genetic diseases, which nowadays is hampered by the difficulty of evaluating the effect of Variants of Unknown Significance (VUS). In-silico predictors represent the gold standard for the clinical interpretation of variants identified through sequencing approaches; unfortunately, even the most novel tools are highly inaccurate, resulting in the unconcluded classification of most observed mutations, thus VUS. This leads to wrong diagnoses and inadequate clinical management of patients carrying genetic disorders.

To address this point, Mutagenesis by Integrated TilEs (MITE) is a novel saturation mutagenesis approach that allows the quick testing of thousands of protein variants in a single high-throughput functional assay coupled with Next-Generation Sequencing (NGS). We applied MITE to mutagenize two regions (the entire SAM domain and a portion of DNA Binding Domain) of P63 (~2300 variants), a transcription factor which, besides an oncogenic potential, is a key regulator of skin development. To follow the biological activity of each generated variant, we set up efficient fibroblasts to keratinocytes conversion induced by P63-KLF4 overexpression. By separating converted and non-converted cells based on the specific keratinocyte antigen ITGβ4 expression, we could study the enrichment of each variant in the two populations and characterize the mutational hotspots.

To expand such a method to any other disease-driving gene, we developed a strategy to couple this technology with single-cell RNAseq; this allows measuring at the single-cell level the specific transcriptional signatures induced by any possible missense mutation in the desired gene of interest and the mutation itself.

In summary, these results will represent an advancement in functional genomics applied to genetic disorders, providing the scientific community with a robust operating platform for genetic and drug discovery studies for any possible disease-driving gene.

#452 - NIG: NETWORK FOR ITALIAN GENOMES

Requested topic(s): PANGENOMES AND GENOMIC DIVERSITY

Requested presentation type: Oral presentation

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Abstract
Network for Italian Genomes is a platform that supports genomic and genetic research by creating a reference Italian genome based on metadata analysis.

Genetic data and phenotypic datasets have been collected for 18173 Italian individuals through the collaboration between NIG and major Italian universities as well as hospital institutions (Ospedale Pediatrico Bambino Gesù, Ospedale San Raffaele, ASST Ospedale Papa Giovanni XXIII, Università degli Studi di Bologna, Università della Campania "Luigi Vanvitelli", Università degli Studi di Pavia, Università degli Studi di Siena, Università degli Studi di Torino, Università degli Studi di Tor Vergata, Università degli Studi di Trieste).

In order to enable computational resources for genome sequencing analysis produced by the collaborators, NIG employs the CINECA infrastructure.

The metadata analysis conducted by each centre will provide a characterization of sequence variants in Italian individuals (differentiated through Principal Component Analysis) and stratified by sex and age. Variant calling will be performed using the CINECA joint call pipeline. The main objective is to define an Italian reference genome with the aim of: i) identifying genes responsible for genetic diseases and susceptibility genes for complex diseases, both in basic and translational research; ii) identifying genetic variants responsible for inter-individual differences in drug response, useful for demographic and forensic purposes; iii) defining new targets for the diagnosis and treatment of genetic diseases.

To join NIG project and become a collaborator of the platform, it is possible to compile the online form available at https://www.nig.cineca.it, providing information about the institution and the number of exomes and/or genomes available for upload, to which a response will be given within a week.

The purpose of a centralized repository is to coordinate data storage on a single platform, to improve access for the biomedical community, while ensuring data privacy and confidentiality.

#292 - DIFFERENTIAL METHYLATION IN ANCIENT AND MODERN PEOPLE SUGGEST EARLY APPEARANCE OF CHRONIC INFLAMMATORY DISEASE

Requested topic(s): TRANSGENERATIONAL INHERITANCE / EPIGENETICS

Requested presentation type: Oral presentation

Abstract Details

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Abstract

Motivation and Aim: Cardiovascular diseases, particularly atherosclerosis, have surged as a leading cause of mortality, linked closely to metabolic syndrome, which includes conditions like arterial hypertension, type 2 diabetes mellitus, and obesity. The shift from a hunter-gatherer to a sedentary lifestyle is deemed crucial in this syndrome, impacting an individual's epigenetic profile and potentially inducing rapid phenotypic changes. This study explores the evolving epigenetic mechanisms underlying atherosclerosis, aiming to identify differentially methylated regions (DMRs) associated with metabolic syndrome and atherogenesis, serving as potential targets for epigenetic editing.

Algorithms and Methods: Addressing the challenge of comparing ancient and modern DNA profiles, the study utilized bone genomes from 3 ancient individuals and 7 modern hunter-gatherers, applying the DamMet algorithm for methylation profiling. A custom Python script compared methylation levels in hunter-gatherers, ancient individuals, and 40 modern subjects. Analysis of modern whole-genome bisulfite sequencing (WGBS) samples identified tissue-specific regions, allowing the comparison of modern atherosclerotic individuals with hunter-gatherers.

Results: The comparison revealed nearly 500 DMRs associated with the atherosclerotic phenotype, predominantly exhibiting hypomethylation in modern individuals. Notably, two genes—HDAC4 and IGF1R—displayed hypermethylation, indicating potential silencing. Gene ontology analysis unveiled correlations with insulin response, energy metabolism, adipogenesis, and systemic inflammation.

Conclusion: The study validated the DamMet algorithm and identified optimal parameters for ancient samples, uncovering significant differences in non-tissue-specific methylation of promoters associated with metabolic syndrome and atherosclerosis formation. Further investigations, including the analysis of the modern human bone methylome in atherosclerotic subjects, are crucial to define tissue-specific methylation changes. The identified DMRs present potential targets for epigenetic editing in cells, providing insights into their role in atherosclerosis development.

Acknowledgements: This work received support from the Russian Science Foundation (Grant # 23-65-10014).

#360 - KETOGENIC DIET THERAPIES FOR DRUG-RESISTANT EPILEPSY MIGHT AFFECT ION CHANNELS ACTIVITY THROUGH THE COMBINATION OF BOTH EPIGENETIC CHANGES AND SPLICING EVENTS

Requested topic(s): TRANSGENERATIONAL INHERITANCE / EPIGENETICS

Requested presentation type: Oral presentation

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Abstract

Background

Diet is among the most relevant epigenetic modulators, and increasing evidences suggest that epigenetic changes (DNA methylation, histone acetylation and chromatin remodeling) are also associated with ketogenic diet (KD) therapies. These are considered an effective approach for the management of drug-resistant epilepsy , such as GLUT1 deficiency syndrome (GLUT1-DS; #606777), whereas its underlying mechanisms remain elusive.

After the confirmation of the metabolic status, we aimed to explore the transcriptional dysregulations of two SLC2A1-mutated pediatric patients before and 6/8 months after the treatment with KD and to also assess alterations in the methylome profiling.

Methods

FIA-MS/MS platform was carried out for the analysis of 11 amino acids and 31 acyl carnitines. Bulk RNA sequencing was performed using Illumina Stranded Total RNA Pre kit and sequenced on NS500. Differential expression analysis was conducted using R Package DESeq.2 and gene set enrichment analysis (GSEA) was performed using Cluster Profiler. Analysis of alternative splicing and isoform switches was implemented using IsoformSwitchAnalyzeR. Methylation sequencing was carried out on PromethION 2 Solo platform and data analysis was showed using Remora algorithm.

Results

Metabolite analysis has confirmed the ketosis status in two patients in diet (increase of C2 and C4OH). RNA-seq data highlighted a significant splicing change in GLUT1-DS children under KD treatment identifying the snRNA U1 up-regulated before the therapy. In addition to amino acids metabolism, translation and mitochondrial activity deregulated, when performing GSEA with Reactome a strong epigenetic signature was also observed: histones deacetylation and DNA methylation resulted down-regulated. Molecular function analysis showed a down-regulation in voltage-gated ion channel and passive transmembrane transporter activities. Moreover, analysis for 5mC and 5hmC profiles identified differentially methylated CpG sites before and after KD.

Conclusions

The observed epigenetic changes related to KD treatment might influence the alternative RNA processing of voltage-gated ion channels also by epigenetic splicing code.

#389 - A CLINICAL READY, LONG READS SOLUTION FOR SEQUENCING HUMAN MICROBIOTA

Requested topic(s): MICROBIOME AND METAGENOMICS

Requested presentation type: Poster presentation

Abstract Details

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Abstract
The human microbiome is characterized by several dynamic microbial communities that colonize different anatomical locations in the human body, establishing a symbiotic equilibrium with the host. Evidence of variations in microbial community composition and function have been found to occur substantially over the life of an individual, playing a pivotal role in promoting human health. Consequently, perturbations in the human microbiome can cause or exacerbate different kind of diseases.

Several studies have identified multiple factors that influence the human microbiome composition. Among these, we can certainly include disease state, genetics, body site, age, pharmacological treatments.

Monitoring how human microbiome changes across time can be helpful in determining the repercussions and downstream influences of these factors on our biota. In this perspective, we developed and validated a long-reads-based approach to identify both bacterial and fungal strains with Next Generation Sequencing (NGS) on Oxford Nanopore Technologies (ONT) sequencers. ONT technology allows to sequence long DNA fragments, therefore obtaining continuous sequences of targeted genes, fundamental aspect when dealing with metagenome samples.

The kit allows to target the entire 16S, 18S and ITS1+5.8S+ITS2 genes with an amplicon-based approach. The sample multiplexing and desired coverage generate libraries of up to 64 samples hosted on a MinION flow cell.

The kit was validated on ATCC standards (skin standard MSA-1005, gut standard MSA-1006, vaginal standard MSA-1007) with NGS sequencing on a MinION (flow cell chemistry R10.4.1, Super-accurate basecalling). The data analysis was carried out on One Codex platform using the ATCC Microbiome Standard 2021 (WGS) as reference.

Our validation results demonstrated the ability of the kit to specifically identify the different strains contained in the ATCC standards. A total of 89.0%, 87.7% and 88.6% of reads (respectively skin, gut and vaginal standard) mapped to true positives in the controls. Furthermore, the detected relative abundances of each strain were coherent with the expected ratios.

In conclusion, this study highlights the robustness and precision of our long read based kit in detecting the bacteria and fungi species The kit is currently under external validation at both private and public centers and hospitals, in order to spread human microbiome monitoring.