The genetic basis of Growth Hormone Deficiency in a Portuguese Patient Cohort

Ana C., Ribeiro[a,b,*]; Melanie, Makhlouf[a]; Najeeb, Syed[c]; Manuel C., Lemos[b,#]; Luis R., Saraiva[a,#]

[a] Translational Medicine Division, Sidra Medicine, Doha P.O. Box 26999, Qatar
[b] CICS-UBI, Health Sciences Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal
[c] Integrated Genomics Services, Sidra Medicine, Doha P.O. Box 26999, Qatar

Growth Hormone (GH) deficiency (GHD) is a rare disorder which in the absence of treatment can result in growth failure and severe short stature. An increasing number of idiopathic cases are becoming recognized to have a genetic origin, with variants identified in genes that affect the development and/or function of the pituitary gland. In this study, we aimed to characterize the genetic basis of idiopathic GHD in a cohort of 150 Portuguese patients. To this end, we performed Whole Exome Sequencing (WES), followed by the bioinformatic analyses using standard pipelines. Putatively pathogenic variants were filtered in genes biologically related to GH axis, pituitary development, and pituitary-related disorders. A total of 505 genes were included in the analysis, including 45 genes already known to be associated with GHD. The triage of variants was done by keeping all non-synonymous variants located in the exons and intron-exon boundaries, with a mean allele frequency less than 0.1% in the general population, absent from the WES data from a Portuguese healthy cohort (N=50), and with a CADD score higher than 20. In total, we obtained 641 variants, including 69 variants in 25/45 GHD-related genes, and 572 variants in the 259/460 additional genes. The top six genes mutated in these patients were CEP290, LRP2, VPS13B, HERC2, CDH23, and GLI2. In conclusion, the WES analysis of Portuguese GHD patients identified 641 variants, and top mutated genes could be potential pathogenic candidates in these patients.

* Presenting author
# Correspondence: mclemos@fcsaude.ubi.pt (M.C.L.); saraivalmr@gmail.com (L.R.S.)

Metabolomics reveals distinct metabolic profiles of T-cells during the production of CD19-chimeric antigen receptor (CAR) T-cells

Shana Jacob[a]*, Saroja Kotegar Balayya[a], Asma Al-Sulaiti[b], Jean-Charles Grivel[c] and Cristina Maccalli[b]#

a Advanced Cell Therapy Core, b Laboratory of Immune and Biological Therapy, c Deep Phenotyping Core; Sidra Medicine; Doha; QATAR

Chimeric Antigen Receptor T-cells have improved the treatment of advanced hematological malignancies and are being developed for the treatment of some types of solid tumors. The genetic modifications and cellular expansion *ex-vivo* of T-cells that are needed to produce efficient CAR-T-cells are dependent on their metabolic status both during manufacturing and the effective phase in the tumor microenvironment. Metabolomics plays a central role in the characterization of T-cells and CAR-T cells and can distinguish cells that have been subjected to different manufacturing processes.

T-cells isolated by magnetic separation from mononuclear cells of umbilical cord blood were activated *in vitro* for 48 hours using anti-CD3 and -CD28 monoclonal antibodies conjugated to either magnetic beads or a polymeric nanomatrix (T-cell TransAct; Miltenyi). The cells were then transduced with lentiviral vectors encoding for CD19-CD28ζ or -4-1BBζ CARs followed by coculture with $CD19^+$ Raji cells or $CD19^-$ K562 cells or directly purified without a coculturing step. Triplicate biological replicates of the T-cells from the different groups of CAR-T cells (N=18) were then subjected to metabolomics analysis using liquid chromatography-high resolution mass spectrometry. Relative quantification and annotation were achieved for 199 metabolites in all groups.

When comparing the activation step, beads vs TransAct, and then stimulation with Raji cells, it was found that the T-cells activated with beads indicated greater growth and were more metabolically anti-tumor responsive (asparagine; FC=138; p=0.0019). Following this, comparisons was made between beads-activated T cells transduced with two different vectors encoding for CD19-CAR (CD28 ζ vs. 41BB ζ). The CD19-CD28ζ-CAR T cells showed low metabolic activity when cocultured with both Raji and K562 cells. The CD19-4-1BBζ-CAR T cells showed evident metabolic differences after coculture with Raji and K562 cells. When CAR T-cells transduced with 41BB and cocultured with Raji were compared with untransduced T-cells also cocultured with Raji, changes of 53 metabolites indicated a decreased disease activation state for solid tumors as indicated by Ingenuity Pathway Analysis (z-score -2.065, p=2.93E-17). This indicates that anti-tumor function can be identified based on the successful engineering of T-cells with CARs.

Lipid metabolism is altered in zebrafish model of *MYBPC3* hypertrophic cardiomyopathy

Shana Jacob[a]*, Saroja Kotegar Balayya[a], Waseem Hasan[b], Doua Abdelrahman[b], Jean-Charles Grivel[c], Mohamed A Elrayess[d], Khalid Fakhro[e,f,g], and Sahar Da'as[b,g #]

a Advanced Cell Therapy Core, Sidra Medicine, b Zebrafish Core Facility, Sidra Medicine, c Deep Phenotyping Core, Sidra Medicine, d Biomedical Research Center, Qatar University, e Human Genetics, Sidra Medicine, f Weill Cornell Medical College - Qatar, g College of Health and Life Sciences, Hamad Bin Khalifa University

Mutations in the cardiac myosin-binding protein C3 gene (*MYBPC3*) are the most common genetic cause of hypertrophic cardiomyopathy (HCM). The molecular mechanisms leading to HCM remain poorly understood.

Seven replicates of zebrafish larvae (n=150) from control (CO), heterozygous (HT), and homozygous (HM) groups, were analyzed for lipidomic and metabolomic profiles using liquid chromatography-high resolution mass spectrometry.

The untargeted analysis revealed 639 annotated metabolites that were used for statistical analysis. Partial Least Squares Discriminant Analysis (PLS-DA) revealed the distinct clustering of the three groups with 45.4% of variability explained by the first two components. A clear separation was made in the first component that distinguished the HM group from the others. The important features that led to this separation were several classes of lipids. Among these lipids, higher abundance levels of acylcarnitines were noted in the HM group compared to CO and HT groups suggesting a higher use of beta-oxidation of fatty acids as opposed to glucose metabolism as an energy source for cellular activities. In the myocardium, this high dependence on fatty acid oxidation for energy is reported to lead to hypoxia, myocardial systolic dysfunction, and myocardial hypertrophy.

In addition, pathway enrichment analysis revealed significant changes in glycerophospholipid metabolism when directly comparing HM and HT groups (FDR p=1.3E-4) with 11 of 38 metabolites being detected in the KEGG pathway. Of these the greatest differences were among phosphatidylcholine (p=4.3E-5), lysophosphatidylcholine (p=2.2E-6) and glycerophosphatidylcholine (p=1.7E-5).

This study provides new insights into the lipid and metabolite profiles contributing to HCM pathogenicity at the early stages of cardiac development. The findings highlight the potential for development of new HCM therapeutics targeting metabolic alterations.

The Unique Q493R Mutation Drives Interfacial Interactions in the SARS-CoV-2 Omicron Variant S1-RBD:ACE2 Complex

Angelin M Philip[1*], Wesam S Ahmed[2], Kabir H Biswas[2,#]

Affiliation:
[1]Division of Genomics and Translational Biomedicine, College of Health & Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Doha – 34110, Qatar
[2]Division of Biological and Biomedical Sciences, College of Health & Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Doha – 34110, Qatar

Keywords: ACE2; COVID-19; Omicron; Molecular Dynamics Simulation; Infectious Disease; SARS-CoV-2; S1 Spike Protein;

Abstract
The SARS-CoV-2 Omicron variant containing 15 mutations, including some that have been found in other variants such as the N501Y mutation and a unique Q493R mutation, in the receptor binding domain number of the S1 spike protein (S1-RBD). While comparison with previously reported mutations in other variants provide some insights, the molecular mechanism underlying the increased rate of Omicron infections is not yet completely understood. Here, using structural modelling and molecular dynamics (MD) simulations, we show that the mutations in the Omicron S1-RBD result in an increased interaction with ACE2. Specifically, we generated a model of the ACE2-S1-RBD complex variant containing all mutations associated with the Omicron variant and performed two, 100 ns long all atom, explicated solvent MD simulations. Comparative analysis of the Omicron ACE2-S1-RBD complex trajectory with that of the WT complex revealed a substantial reduction in the Cα-atom fluctuation in the Omicron S1-RBD without much alteration in the overall structural dynamics of the protein. Further analysis revealed an increase in the number of hydrogen bond (H-bond) formation, along with increased van der Waal and electrostatic interactions, in the Omicron ACE2-S1-RBD complex in comparison to the WT complex. Residue level analysis revealed an alteration in the interaction between several residues including a switch in the interaction of ACE2 D38 from S1-RBD Y449 in the WT complex to the mutated R residue (Q493R) in Omicron complex. Furthermore, the observations made in the Omicron+Q493R (omicron without Q493R mutation) revealed the significance of the Q493R mutation unique to the Omicron variant. Thus, these computational results provide significant mechanistic insights into the increased interaction of the Omicron S1-RBD with ACE2, and thus, the increased infectivity of the variant. Current study will aid in the development of improved therapeutic inhibitors of S1 spike protein-ACE2 interaction such as antibodies and synthetic peptides and proteins.

Elevated Salivary alpha amylase is associated with reduced levels of adiposity markers and inflammatory markers CRP and TNF alpha and increased levels of anti-inflammatory protein Adiponectin in obese non-diabetic Qatari women

Neyla S. Al Akl [1*], Olfa Khalifa [1], Mohammad Habibullah[2], Abdelilah Arredouani[1,3#]

[1]Diabetes Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar

[2] College of Medicine, Qatar University, Doha, Qatar

[3]College of Health and Life Sciences, Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar

Obesity has been associated with increased risk of several complications, including type 2 diabetes (T2D) and cardiovascular diseases (CVD). The prevalence of obesity is on the rise worldwide, and Qatar is one of the most affected nations with about 80% overweight or obese adults. Therefore, identifying biomarkers that can predict obesity and its co-morbidities is of paramount clinical importance to develop effective preventive and management strategies. Recently, copy number variations (CNVs) in the salivary α-amylase (sAA) gene AMY1 have been linked to obesity predisposition and resistance to insulin in adults and children. Yet, the association between sAA and cardiometabolic risk factors has not been fully decoded. Also, only few studies have explored the relationship between sAA activity and other cardiovascular parameters and other risk markers risk such as chronic low-grade inflammation.The aim of the study is to investigate the association between serum sAA activity and adiposity markers associated with risk of cardiovascular disease activity and markers of chronic low-grade inflammation. Serum samples and clinical data of 1500 adult non-diabetic overweight/obese participants were collected from Qatar Biobank (QBB). Plasma salivary alpha amylase (psAA) and C reactive protein (CRP) levels were estimated with an autoanalyzer. Serum cytokines and adipokines, including TNF-α, IL-6, IL-1β, MCP-1, leptin and adiponectin, of 228 patients were quantified using the bead-based multiplex assay. The association between the sAA and the adiposity indices and low-grade inflammatory proteins and cytokines was assessed using pearson's correlation and adjusted linear regression. The different adiposity markers body mass index, waist circumference, hip circumference, body fat%, body adiposity index, cholesterol and HDL were significantly and inversely associated with psAA in women. Adiponectin, protein with an anti-inflammatory role was significantly and positively associated with psAA in women. CRP and TNF-α, inflammatory proteins, were significantly and negatively associated with psAA in women. Our findings suggest that elevated sAA plays a role in fostering anti-inflammatory and anti-cardiovascular risk factors and hinders the levels of pro-inflammatory proteins in women.

Epithelial Organoids: Establishing a Patient-Derived Culture System to Tackle Monogenic Disorders of the Mucosal Barrier

Ahmad, Al-Shaibi[a]; Bernice, Lo[a].
[a]: Research Branch, Sidra Medicine, Doha, Qatar

With the emergence of precision medicine, demand for additional patient-derived culture models to evaluate disease pathogenesis and test potential therapeutic approaches has been increasing. In inflammatory bowel disease, much of the therapeutic attention has been focused on managing immune-related symptoms with reduced attention to other crucial components of GI homeostasis like the mucosal barrier. With the recent discoveries of monogenic defects in the function of the mucosal barrier, such as our recent publication on AGR2 deficiency, modeling this disease paradigm *in vitro* is increasingly important both as a tool to discover and validate new disease-causing variants and to test medicines more targeted towards managing mucosal barrier function. Epithelial organoids as a patient-derived culture model fit the role perfectly and work well in compliment with existing cell culture models and animal models. We have been successful over the last few months in establishing a 3D epithelial organoid system derived from patient biopsies using published methods. Epithelial stem cells and crypts are isolated via chelation and mechanical disruption from donor biopsies then cultured in Matrigel in 3D under conditions optimized for either stem cell proliferation or differentiation while maintaining a stem cell niche. Organoids can then be phenotyped using any number of high throughput methods to observe and experiment on their different cell niches. Our self-imposed criteria for establishing the technique dictated that organoids must be successfully grown, propagated, and differentiated from biopsies freshly isolated or cryopreserved as well as from previously grown and cryopreserved organoids. We have been successful in doing so with colonic organoids showing differentiation of goblet cells and mucus production. Establishing this system and paving the way for organ chips will accelerate the discovery and validation of local and regional variants affecting mucosal barrier function, while providing a precision medicine tool that can directly improve the quality of care.

iPSCs derived from insulin resistant offspring of type 2 diabetic patients show increased oxidative stress and lactate secretion

Bushra Memon[a,b], Ahmed K. Elsayed[b], Ilham Bettahi[c,d], Noor Suleiman[c], Ihab Younis[e], Fares El-Ejeh[f], Eman Wehedy[a,b], Abdul Badi Abou-Samra[c], Essam M. Abdelalim*[a,b]

[a] *College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Education City, Doha, Qatar.*

[b] *Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, PO Box 34110, Doha, Qatar.*

[c] *Qatar Metabolic Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar.*

[d] *Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar.*

[e] *Biological Sciences Department, Carnegie Mellon Institute in Qatar, Qatar Foundation, Doha, Qatar.*

[f] *Cancer Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, PO Box 34110, Doha, Qatar.*

Abstract

Insulin resistance (IR) is caused by both genetic and acquired factors, however, the genetic factors are not well understood. The insulin-resistant first-degree relatives of type 2 diabetic (T2D) patients have the highest genetic predisposition to T2D. Induced pluripotent stem cells (iPSCs) are excellent tools for disease modeling as they can retain the genetic imprint of the disease. Therefore, in this study, we aimed to investigate the genetic perturbations associated with IR in the offspring of T2D patients, by using iPSC technology. iPSCs were generated from lean, IR offspring of T2D patients (IR-iPSCs (4 subjects)) as well as from insulin-sensitive (IS-iPSCs (3 subjects)) individuals, who were categorized based on hyperinsulinemic-euglycemic clamp. All iPSCs were fully characterized for pluripotency and had normal karyotyping. Transcriptomics revealed that the IR-iPSCs have increased oxidative stress and a heightened response to hypoxia, indicated by accumulation of reactive oxygen species and a high susceptibility to H_2O_2-induced apoptosis. Moreover, the IR-iPSCs also had amplified levels of lactate secretion compared to IS-iPSCs under normal conditions. Intriguingly, IR-iPSCs showed increased phosphorylation of AKT upon activation with 100 nM insulin compared to IS-iPSCs. iPSCs were then differentiated to mature hepatocytes and differentially expressed proteins were assessed using the Olink platform for proteomics. OLink assay for IR-hepatocytes indicated that markers regulating hepatic function, glucose metabolism, glycosylation, autophagy, inflammation, and stress or those associated with IR and T2D were differentially expressed compared to IS-hepatocytes (n=6 per IS and IR). Our results also identified that key markers affecting hepatocyte development and function like p53, GALNT2, DNAJB1 and ATG4A had high levels under serum-starved conditions in the IR-hepatocytes, however, showed no further increase upon insulin-treatment. Additionally, the IR-hepatocytes exhibited a blunted glucose uptake capacity upon insulin treatment when compared to the IS-hepatocytes, highlighting their diminished insulin-responsiveness. Overall, our IR-iPSC model can be employed for T2D modeling and drug screening studies that target genetic perturbations associated with IR in individuals with a high risk for T2D.

New evidence for CSRP2 intron retention in acute lymphoblastic leukemia (ALL)

Vahid Moghimi[a]*, Azin Keshavarz[a], Reza Alemohammad[a], Saeed Khatami[a], Moein Farshchian[a#]

[a] Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran.

Moein.Farshchian@mail.um.ac.ir

Cysteine And Glycine-Rich Protein 2 (CSRP2) is a cysteine-rich amino acid sequence motif (LIM) with putative zinc-binding activity, which regulates the mesodermal commitment pathway. We used RNA-seq to study transcriptome profiling of 10 pediatric acute lymphoblastic leukemia (ALL) and 2 non-leukemic patients (BioProject: PRJNA589314) to profile different splicing patterns in Iranian patients.

We found delta PSI ~60%, which showed intron retention on target exon 12:76860414-76863175 of CSRP2 transcript (ENST00000548783). Further inspection of this locus showed the presence of H3K27Ac sites near this highly conserved noncoding region, and probable transcription start site (TSS) peaks were observed based on FANTOM project reports.

Although we could not confirm a specific functionality for this region, frequent different alternative splicing isoforms of these genes may indicate a probable internal promoter upstream of this intronic region. Taken together, we suggest that further studies are required to shed light on the regulatory roles of this intron retention in promoting pediatric leukemia.

Genetically encoded, BRET-based molecular crowding biosensors

Asfia Sultana[1], Anupriya M Geethakumari[1], Kabir H Biswas[1,*]

Affiliation: [1]Division of Biological and Biomedical Sciences, College of Health & Life Sciences, Hamad Bin Khalifa University, Qatar Foundation, Doha – 34110, Qatar

Abstract

Molecular crowding is pervasive within cells and is known to affect several aspects of biochemical transformations, including enzymatic reaction rates and protein conformation, and thus, cellular physiology, within them. To study the impact of molecular crowding, a range of tools and assays have been developed previously. However, these have been restricted to either monitoring the effect of molecular crowding on either biochemical reaction rates or protein conformation, but not both, simultaneously. Here, we have engineered genetically encoded, Bioluminescence Resonance Energy Transfer (BRET)-based biosensors for monitoring the effect of molecular crowding on enzymatic rate as well as protein conformation, simultaneously. The biosensors were generated by sandwiching rigid $(EAAAK)_3$ and flexible $(GGGGS)_3$ repeats of amino acid sequences, in between the mNeonGreen (mNG; BRET acceptor) and nanoLuc (NLuc; BRET donor) proteins. We utilized the sensors for monitoring the impact of polyethylene glycol (PEG; a range of molecular weights was used), a crowder that has been used extensively in molecular crowding experiments, on the conformation, as reported by the efficiency of resonance energy transfer (BRET), and the biochemical rate, as reported by the bioluminescence from NLuc. These experiments revealed a biphasic response to molecular crowding on the biochemical rate of NLuc while an enhanced impact on the conformation of the rigid biosensor compared to the flexible biosensor. The sensors developed here can be used for monitoring the rate of biochemical reaction and protein conformation in living cells, especially under disease conditions such as those that result in increased intracellular molecular crowding.

Serum untargeted metabolomics reveals alterations in pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and sphingolipid metabolism pathways in diabetes-related cognitive decline.

Abdelilah Arredouani[1,2,✉] , Rayaz Malik[3], and IDRP-diabetes group[1]

[1]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.
[2] College of health and life sciences; Hamad Bin Khalifa University; Qatar Foundation, Doha, Qatar.
3. Weill Cornell Medicine-Qatar, Qatar Foundation, Doha, Qatar.

Background. The pathogenesis of Diabetes-Related Cognitive Decline (DRCD) remains elusive, and the lack of effective markers makes its early identification problematic.

Aim. Unravel the diabetes-driven metabolic alterations that raise the risk of DRCD.

Methods. We included 46 DRCD patients and 26 healthy controls (HC). We used untargeted metabolomics profiling and multivariate statistical analysis to identify differentially expressed metabolites and their related metabolic pathways. We assessed the diagnostic accuracy of key metabolites with ROC analysis. Local IRBs approved the study.

Results. At a fold change >2, we identified 61 up- and 24 down-regulated metabolites in DRCD patients relative to HC subjects. The two groups were clearly separated using OPLS-DA (figure 1a). 50 significantly altered serum metabolites (VIP values from the OPLS-DA > 1; FDR <0.05) were selected for pathway analysis. We identified 3 significant pathways ($p<0.05$), including pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and sphingolipid metabolism. Logistic regression-based ROC analysis using four metabolites (FC >2; $p<0.05$) and 10-fold cross-validation returned a model with an AUC of 0.906 (95%CI: 0.825-0.988), a sensitivity of 0.913 (95% CI: 0.913-0.994), and a specificity of 0.808 (95%CI 0.656-0.959) (figure1b). The four metabolites are myristolamide (14:1), 12-HHTrE, heme, and salicylate. In comparison to HC, the levels of myristolamide were significantly lower in DRCD, while the levels of the others were significantly higher.

Conclusion. The disorders of pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and sphingolipid metabolism pathways could be promising targets for DRCD prevention. On the other hand, differentially expressed serum metabolites are potential biomarkers for disease monitoring and personalized medication complementary to the existing clinical modalities. Further studies are warranted in bigger cohorts.

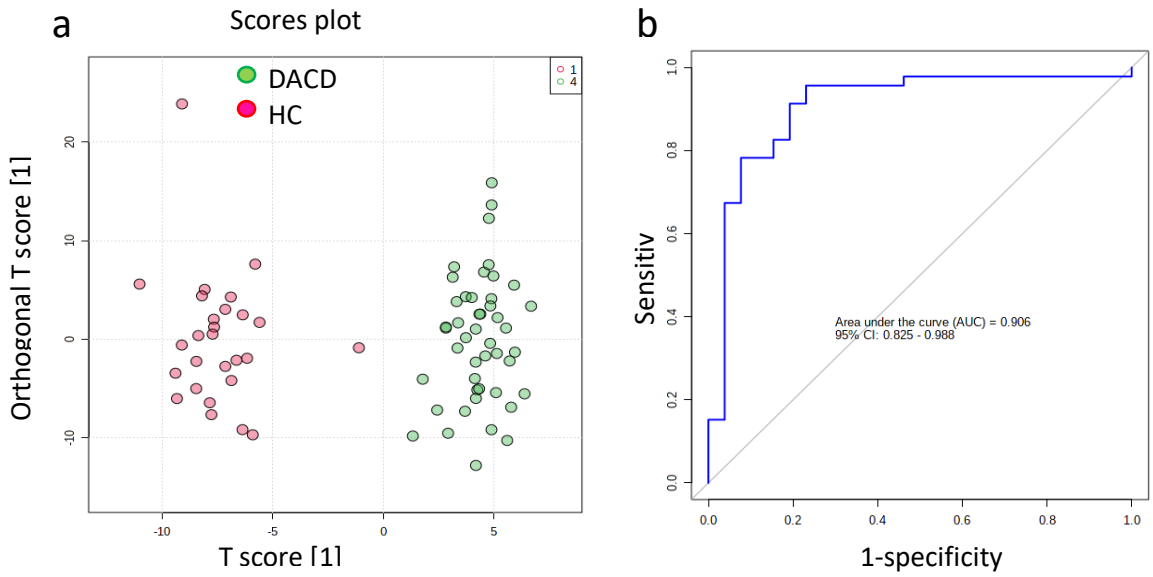

Figure 1. **a)** OPLS-DA score plot of healthy controls (pink) vs DRCD patients (green). **b)** logistic regression-based ROC curve using 4 metabolites

Omouma: A Prospective Mother and Child Cohort at Sidra Medicine to identify Early Predictive Biomarker for Pregnancy Complications

Marwa Saadaoui1, Manoj Kumar[1], Duaa Ahmed Elhag[1], Dalia ABubaker [1], Selvasankar Murugesan[1], Shaikha Al Abduljabbar[1], Dalia ABubaker [1], Yassin Fagier[2], Ortashi Osman[2], Hala Abdullahi[2], Ibrahim Ibrahim[2], Medhat Alberry[3], Anthony Abbas[3], Sawssan R. Ahmed[4], Mohamed A. Hendaus[5], Karim Kalache[3], Abdulkadir Wagley[6], Khawla Ismail[6] , Homaira Karim[6], Nusrat Fazal[7], Ashok Kumar Oliparambil[6], Ghada Mohiyiddeen[6], Sara Buhmaid[6], Agnes Despi[6], Annalisa Terranegra[1], and Souhaila Al Khodor[1*]

[1]Research Department; [2]Obstetrics and Gynecology; [3]Maternal Fetal Medicine; [4]Psychiatry Department; [5]Pediatrics, [6]Women's Services Clinical Group, Sidra Medicine Doha, Qatar

*Corresponding author: Dr. Souhaila Al Khodor (salkhodor@sidra.org)

Background: Pregnancy is a critical "formative period" that has a significant impact on an individual's health trajectory from the fetal life to adulthood. Because pregnancy complications are multifaceted, finding markers that can predict them is critical. The objective of this project is to create a longitudinal mother-baby cohort that will help in the identification of predictive biomarkers for adverse pregnancy outcomes, early life determinants, and their impact on child health.

Material and analysis: We aim to recruit 1000 pregnancies starting from their first trimester (6-14 weeks of gestation) from Sidra hospital and clinics. So far we have recruited 52 pregnant women. We will follow with them every trimester until delivery and one year post -partum. For each mother/newborn pair, a complete dataset will be collected, including anthropometric, physiological and biochemical measurements, medical interventions, pregnancy and delivery details. In addition, various biological samples from the pregnant woman and her baby will be collected. Maternal and neonatal health, including mental health and perinatal growth, will be recorded using a combination of questionnaires, interviews, and medical records. To identify early predictive biomarkers for pregnancy complications, downstream sample processing including microbial profiling, vaginal immune response, blood transcriptomics, epigenomics, and metabolomics, will be performed

Results and discussion: This study will help in the prediction of pregnancy complications and neonatal health outcomes. By comparing the longitudinal profile of pregnant women with adverse outcomes or complications to their matched controls (full-term pregnant women), we are aiming to identify specific predictive biomarkers for the early prediction of pregnancy complications. Additionally, this cohort could also be used to investigate how familial, socioeconomic and lifestyle factors interact with genetic and environmental factors to influence health outcomes and achievements later in life. These findings will hold promise for the diagnosis and precision-medicine interventions to reduce the health burden of complicated pregnancy.

Treatment with pH responsive-DSF-loaded niosomes reduced cancerous phenotype in oral squamous carcinoma cells

Melika Zangeneh Motlagh[1,2*], Zohreh Mirlavasani [3], Mandana Kazem Arki[3], Iman Akbarzadeh[3], Pouyan aminishakib[1,4], Nikoo Hossein Khannazer [3], Nazanin Mahdavi[1], Massoud Vosough[1,5#]

1. Department of oral and maxillofacial Pathology, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Gastroenterology and Liver Diseases Research Center, Research, Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Pathology, Cancer Institute Hospital, IKHC, Tehran University of Medical Sciences, Tehran, Iran.
5. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Stockholm, Sweden

Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancer, and over the last decade its' incidence has increased by 50%. The encapsulation of components in niosomes (non-ionic surfactant-based vesicle) as nano-sized carrier systems has been proposed as they improve the solubility, stability, and bioavailability of drugs. Herein, an optimum formulation of Disulfiram(DSF)-loaded niosomes was developed for the treatment of OSCC to reduce drug doses and improve the poor stability of DSF in the OSCC environment. The Design Expert Software was utilized to optimize the particles in terms of size, polydispersity index (PDI), and entrapment efficacy (EE (%)). Acidic pH increases the release rate of drugs from these formulations. The size, PDI, and EE of niosomes were more stable at 4 °C compared to 25 °C. 3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay showed that the IC50 of synthesized niosomes is the lowest of all the groups tested.

Keywords: Niosome drug delivery, oral squamous cell carcinoma, differentiation therapy

Identification of prognostic and predictive immune and gene signature in breast cancer for precision oncology

Mariam AL-Muftah[*#], Fares AL Ejeh - Translational cancer and immunity center, Qatar Biomedical Research Institute, Hamad bin Khalifa University, Qatar Foundation, Qatar

Breast cancer (BC) is the most diagnosed cancer worldwide and the leading cause of cancer-related deaths in women. In Qatar, in 2020 the percentage of BC within all cancers diagnosed in the 35-54 age group was higher than the global percentage. Younger age of diagnosis is often a characteristic of triple negative breast cancer (TNBC) that accounts for 15-20% of BCs diagnosed. TNBC is highly aggressive, associated with higher risk of recurrence and lacks effective therapies. Due to its immunogenic nature, TNBC has been a candidate for cancer immunotherapy. Several immunotherapeutic antibodies targeting the immune checkpoint proteins PD-1 or PD-L1 have shown promising improved efficacy and were recently approved by the Food and Drug Administration (FDA). Nevertheless, a low frequency of patients responds under current eligibility criteria and half of those patients develop resistance to immunotherapy, highlighting the need for more reliable prognostic and predictive biomarkers for patients' eligibility to immunotherapy. Methylation plays a role in modulating tumor immunity leading to immune evasion and resistance to immunotherapy and is being under investigation as a predictive biomarker for immunotherapy in other solid tumors. We set to identify methylation-associated biomarkers that have biological and prognostic importance in TNBC patients. We conducted in silico analysis on the The Cancer Genome Atlas BC dataset and identified a unique 30-gene signature in TNBC consisting of genes that are targets of the histone methyltransferase EZH2. Higher expression of the 30-gene signature is significantly associated with improved survival in TNBC patients and in inversely associated with immune signatures that contribute to immune evasion. We hypothesis that the 30-gene signature holds important biological and prognostic information. Planned studies are underway to validate the 30-gene signature in a patient cohort in Qatar in predicting benefit to immunotherapy and to investigate its significance to the development of resistance to immunotherapy.

Word count: 300 words (limit 300)

Abstract applicability to PMFG 2022 themes

☒ Advances in Innovative Therapies for Precision Medicine
☒ Big data, new technologies, and translational genomics
☐ Animal, cellular, and organoid models for human disease modeling

Genetic analysis of six families using whole exome sequencing identified nine candidate genes for autism spectrum disorder in the Qatari population

Vijay Gupta[a]*, Afif Ben-Mahmoud[a], Ahmad Kubbar[a], Fouad Alshaban[a], Lawrence W. Stanton[a] and Hyung-Goo Kim[a#]
a-Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar. * Presenting author # Corresponding author.

Autism spectrum disorder (ASD), a neurodevelopmental illness that affects children at an early age with a global prevalence of 1%, is diagnosed based on clinical features such as social impairment, repetitive behaviors, and restricted interests. ASD is genetically heterogeneous and the genetic etiology of ASD in 20-60% of autistic people remains unknown. Next generation sequencing (NGS) technologies such as exome sequencing and genome sequencing have been successful in expanding the total number of ASD genes. In this study, we have performed whole exome sequencing on six syndromic autism trios (affected child and both non-affected parents). Genetic analysis of the sequence data revealed nine candidate ASD genes, seven of which are novel (from chromosomes 2q32.2, 4q12, 5q35.3, 9q31.3, 10q11.23, 12p13.2, Xp22.33) and two previously ASD-associated genes (*ZBTB11* and *KMT2C*). The candidacy of these seven novel candidate genes has been substantiated by the reports describing the genetic variants in those genes. This will require the identification of additional variants in these genes and functional studies to elucidate their pathological roles in neurodevelopmental disorders. The discovery of novel ASD genes described here can not only aid in validating VUSs (variant of unknown significance) of NGS databases to contribute to molecular diagnosis, but also link the signaling pathways implicated in ASD.

Molecular analysis and conformational dynamics of human MC4R disease-causing mutations

Zeyaul Islam[1*], Munazza Tamkeen Fatima[2*], Ammira Sarah Al-Shabeeb Akil[2#] and Prasanna R. Kolatkar[1#]

[*] These authors have contributed equally

[1] Diabetes Research Center (DRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar.

[2] Department of Human Genetics, Translational Medicine Division, Research Branch, Sidra Medicine, Doha, Qatar.

Obesity is a chronic disease with increasing cases among children and adolescents. Melanocortin 4 receptor (MC4R) is a G protein-coupled transporter involved in solute transport, enabling it to maintain the cellular homeostasis. MC4R mutations are associated with early-onset severe obesity, and the identification of potential pathological variants is crucial for the clinical management of patients with obesity. A number of mutations have been reported in the MC4R which are responsible for causing obesity and related complications. Delineating these mutations and analysing its effect on MC4R structure will help in the clinical intervention of the disease condition as well as designing potential drug against it. Sequence based pathogenecity analysis and structure based protein stabilty analysis were done on naturally occuring variants. We used computational tools to analyse the conservation of these mutation on MC4R structure to map the structural variations. Detail structural analysis were carried out for the active site mutations (D122N, D126Y, and S188L) and its influence on the binding of calcium and agonist or antagonist. We performed the molecular dynamics (MD) simulations of the wild type and selected mutations to delineate the conformational changes that provided us the possible reasons for MC4R instability in these mutations. This study provides insight into the potential direction to understand the molecular basis of MC4R dysfunction in disease progression and obesity.

Molecular modelling to study pathogenicity of variants segregating with Mendelian disorders in Qatar

Navaneethakrishnan Krishnamoorthy[a*], Elbay Aliyev[a], Waleed Aamer[a], Ahmet Ozen[b,c], Bernice Lo[a], Khalid Fakhro[a,d,e,#]

[a]Sidra Medicine, Doha, Qatar, [b]Marmara University Division of Allergy and Immunology, Istanbul, [c]Jeffrey Modell Foundation Diagnostic Centre for Primary Immune Deficiencies, Istanbul. [d]Department of Genetic Medicine, Weill Cornell Medical College, Doha, Qatar, [e]College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar

Abstract

In the Precision Medicine Program at Sidra Medicine, when investigating potential disease-causative variants in patients with suspected Mendelian disorders, we found that they are mostly from multidomain proteins. However, majority of these proteins were underrepresented or unresolved in structure prediction platforms and most importantly their functional regions were poorly studied. Recently, we have studied 7 models of proteins to explain functional associations of their variants suspected to cause Mendelian disorders in Qatar. One such example protein is Janus tyrosine kinase3 (JAK3). Understanding normal molecular structure and atomic details of JAK3 is essential to differentiate benign from diseased structure and elucidate pathological mechanisms. JAK3 is involved in several cellular processes such as cell growth, development, immunity via essential signalling events and associated with severe combined immunodeficiency. Although the first 3D model of JAK3 is available, the structure-functional relationships of the domains are unclear. Here, we started with structural evidence-based protein modelling to study all the key functional regions of JAK3. We highlight a few interesting results: 1) many pathogenic mutations (Q501H, A572V, A573V, M576L, A593T, R657Q etc.) were in the pseudokinase domain (PsKD) of JAK3 and other homologous residues V585 (JAK1-V651), L586 (jak2-L611), L587 (Jak1-L653) appear to be functionally relevant to JAK3. 2) ATP binding site of the PsKD shares more structural similarity with JAK2, 3) potential key sites for phosphorylation were identified at the domain-domain interfaces and near the ATP binding site, and 4) key residues at the interface of PsKD and kinase domain were identified that may be involved in the autoinhibition of JAK3. These results might help us to understand the structure-functional relationships of the different domains, the potential role of the key regions and the disease mechanism of new JAK3 mutations.

Primary keratinocytes that are forced into forming spheroids express markers of the hair follicle bulge stem cell niche

Jeremias Frank[b], Thomas Trafoier[b], Amira Zroud[a], Martin Hermann[c], Martin Steinhoff[a, d], Christina Heufler[b], Matthias Schmuth[b], Julia Reichelt[a, b, *, #]

[a]Interim Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar [b]Dermatology, Allergology and Venereology, Innsbruck University, Innsbruck, Austria [c]Department of Anesthesiology and Critical Care Medicine, Innsbruck University, Innsbruck, Austria [d]Department of Dermatology and Venereology, Hamad Medical Corporation, Doha, Qatar [*]Presenting author [#]Corresponding author

Multipotent epidermal stem cells are localized in the hair follicle bulge region. This mostly quiescent keratinocyte stem cell population regenerates the hair follicle, the sebaceous gland, and the interfollicular epidermis during wound healing. Studying these cells in vitro has been challenging because primary epidermal keratinocytes isolated from skin biopsies loose expression of stem cell markers in 2D cell culture rapidly, often before the first passaging. 3D epidermal sheets provide excellent models of the interfollicular epidermis but cannot be used to study the hair follicle bulge niche.

In order to create a model for the hair follicle bulge we established 3D spheroid culture of primary keratinocytes. Following seeding into specialized microwell culture dishes that prevent cell adhesion, keratinocytes formed spheres within 24 hours. A single well in a 6-well microwell plate can be used to produce 5900 uniformly sized spheres. Pulse-chase EdU (5- Ethynyl-2'-deoxyuridine) labelling assays over four days showed that DNA replication and cell proliferation slowed down, and keratinocytes became quiescent within 24 hours after seeding into microwells. Immunofluorescence analysis revealed expression of the stem cell markers keratin (K)15, Sox9 (SRY-Box Transcription Factor 9), tenascin C, p63 and YAP (Yes-associated protein) in the center of the spheres. The basal epidermal marker K5 was expressed throughout whereas the differentiation marker K10 was confined to a few large, flattened cells lining the sphere surface.

The results of our studies demonstrate that cultured keratinocyte spheres exhibit several hallmarks of hair follicle bulge stem cells. The data suggest that these 3D organoids may provide a useful model for multiple applications portending advances in both basic biology and clinical translation. Applications may include drug screening, in vitro modelling of biological processes and diseases, regenerative medicine, and precision medicine correlating drug sensitivity to genomic alterations.

The integration of the genomic and immunological characterization of colorectal and breast cancer cell lines can allow the identification of stem-like features: implications for cancer immunotherapy

Neha Gopinath[1], Alice Turdo[2], Ishita Gupta[1], Rebecca Mathew[3], Harshitha Shobha Manjunath[3], Tanwir Habib[4], Mohammed Toufiq[5], Matilde Todaro[2], Giorgio Stassi[6], Sara Tomei[3], Ira Skvortsova[7], Soldano Ferrone[8,] Cristina Maccalli[1]

[1]Laboratory of Immune Biological Therapy, Translational Medicine; [3]Omics Core, Integrated Genomics Laboratories, Research Department, [5]System Biology Lab, Sidra Medical and Research Center, Doha Qatar.
[2]Cellular and Molecular Oncology Laboratory, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties; [6]Laboratory of Immunological Medicine, Department of Surgical and Oncological Sciences, University of Palermo, Italy.
[4]Weill Cornell Medicine, Doha, Qatar7
[6] Laboratory for Experimental and Translational Radiation Oncology (EXTRO-Lab), Department of Therapeutic Radiology and Oncology, Medical University of Innsbruck and Tyrolean Cancer Research Institute, Innsbruck, Austria.
[7] Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, (MA), USA

Background: The aim of this study is to identify the molecular mechanisms regulating the immunological properties of cancer stem cells (CSCs) isolated from solid tumors.
Methods: Colorectal cancer (CRC; N=15) and breast cancer (BC; N=21) cell lines, including differentiated tumor cells and CSCs, and, for BC cell lines, selected *in vitro* for radioresistance or invasiveness were used for this study. The expression profile of HLA and of the antigen processing machinery (APM) molecules was assessed through flow cytometry. DNA and RNA were isolated from these cell lines. The nCounter platform (Nanostring) was utilized to assess the hybridization with 800 probes for miRNAs and the RNA seq-based transcriptomic profile was also assessed. The methylation profiling of cancer cell lines was investigated through Infinium EPIC arrays (Illumina). In addition, the cell lines treated or not with with immunomodulatory or epigenetic agents to stimulate *in vitro* HLA-matched lymphocytes was utilized to determine the efficiency in eliciting antigen-specific T cell responses.
Results: The expression of HLA and APM (e.g., LMPs, TAP and tapasin) molecules was impaired in CRC and BC cell lines and this phenomenon was more marked in “stem-like” cells as compared to the differentiated tumor cells. The induction *in vitro* of anti-tumor T cell responses through the co-culture of tumor cells with PBMCs correlated with the levels of the expression of HLA and APM molecules by tumor cells. Differential miRNAs, transcriptomic and methylation profiles ($p<0.05$) were identified in either CRC or BC cells with stemness properties vs. differentiated cells, and in different subtypes of cells. These includes genes and their regulators involved in immunological functions. **Conclusions:** These investigations contribute to understand the mechanisms regulating the

immunological properties of tumor cell endowed with variable cell fate and their susceptibility to immunotherapy.

This study was funded by QNRF; grant # NPRP10-0129-170277

Understanding the effect of *GCK* mutation in the development of monogenic diabetes using human iPSC and CRISPR/Cas9 technologies

Yasmin Abu Aqel[1], Racha Chouaib[1], Aldana I. Alnesf[1,2], Idil Ahmed[1], Gowher Ali[1], Ahmed K. Elsayed[1], Zeyaul Islam[1], Sara Al Khawaga[2], Prasanna R. Kolatkar[1,2], Paul J. Thornalley[1,2] Khalid Hussain[3], Essam M. Abdelalim*[1,2]

[1]*Diabetes Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar.*
[2] *College of Health and Life Sciences, Hamad Bin Khalifa University (HBKU), Qatar Foundation, Education City, Doha, Qatar.*
[3]*Division of Endocrinology, Department of Pediatric Medicine, Sidra Medicine, Qatar.*

*****Corresponding Author
Essam M. Abdelalim, PhD
E-mail: emohamed@hbku.edu.qa

Abstract

Glucokinase (GCK, hexokinase IV) phosphorylates glucose to glucose-6-phosphate during glycolysis process in pancreatic β-cells and hepatocytes. This is the rate limiting step in glucose metabolism and enables those cells to respond appropriately to blood glucose level. Mutations in the *GCK* gene could cause either hyperglycemia or hypoglycemia. Heterozygous loss of function mutation causes maturity-onset diabetes of the young 2 (MODY2), while homozygous inactivating mutation leads to permanent neonatal diabetes mellitus (PNDM). Understanding the development of diabetes due to *GCK* mutations is not fully understood due to lack of human models. In the current work, we generated induced pluripotent stem cells (iPSCs) from blood cells of two patients diagnosed with heterozygous and homozygous mutations in the *GCK* gene. The mutations were confirmed in patient's samples using whole exome sequencing (WES) and Sanger sequencing. The iPSC lines were extensively characterized using different approaches. To understand the effect of the mutation, enzymatic experiments were done and our preliminary results showed that the mutated GCK protein is

less stable compared to the wild type (WT) controls although they are both well folded. Furthermore, it showed higher binding ability and affinity to glucose. Moreover, the *in silico* analysis suggested that this *GCK* mutation may affect the binding affinity of GCK with glucokinase regulatory protein (GKRP). To generate isogenic controls, CRISPR/Cas9 knock-in approach was used to correct the mutation in the generated iPSCs. Mutated and corrected iPSC lines were differentiated into pancreatic and hepatic lineages to understand the effect of *GCK* mutations in the development of MODY2 and PNDM. Additional functional studies were performed on both pancreatic β-cells and mature hepatocytes to understand the molecular mechanisms underlying the defects associated with *GCK* mutations. These human iPSC models can provide valuable insights into the underlying mechanisms of monogenic diabetes, which will pave the way towards personalized treatment.

A Novel Homozygous Variant in Homologous Recombination Repair Gene *ZSWIM7* Causes Azoospermia in Males and Primary Ovarian Insufficiency in Females

Shah Hussain[a†], **Shoaib Nawaz**[b,c†*], Ihsan Khan[d], Nida Khan[e], Shabir Hussain[a], Imran Ullah[a], Khalid A. Fakhro[c,f,g], Wasim Ahmad[a#]

Affiliations:

[a] Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan

[b] Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan.

[c] Department of Human Genetics, Sidra Medicine, Doha, Qatar.

[d] First Affiliated Hospital of USTC, Hefei National Laboratory for Physical Sciences at Microscale, the CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Basic Medical Sciences, Division of Life Sciences and Medicine, CAS Center for Excellence in Molecular Cell Science, Collaborative Innovation Center of Genetics and Development, University of Science and Technology of China, Hefei 230027, China.

[e] Department of Obstetrics & Gynecology, Hayatabad Medical Complex, Peshawar, Pakistan.

[f] Department of Genetic Medicine, Weill Cornell Medical College, Doha, Qatar.

[g] College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar.

[†] Contributed Equally

*Presenting Author

#Corresponding Author

ABSTRACT

Infertility is a common, clinically heterogeneous reproductive disorder worldwide with a prevalence of about 15%. To date about 80 genes have been discovered to cause non-syndromic infertility, affecting males and females equally, though traditionally the genetic analysis of each group has been conducted separately. Here, we report the clinical and genetic characterization of a consanguineous family of Pakistani origin with multiple individuals, including male and female, affected with infertility. Males exhibited azoospermia whereas females had primary ovarian insufficiency.

Whole exome sequencing revealed a missense variant (c.176C>T, p.(Ser59Leu)) in the *ZSWIM7* gene which functions in homologous recombination repair. The variant was found in a homozygous form in all affected males and females. To our knowledge, this is the first mutation in *ZSWIM7* to be shown to cause infertility in both sexes, pointing to the utility of large consanguineous families with multiple affected siblings to reveal joint mechanisms affecting human reproduction.

Keywords

Infertility, azoospermia, primary ovarian insufficiency, *ZSWIM7*

PMFG2022

Autism Spectrum Disorder (ASD) in Qatar: Whole genome sequencing of 100 affected families highlights Dominant and Recessive risk genes of ASD

Mona Abdi[1,2*], Elbay Aliyev[2], Brett Trost[3,4], Muhammad Kohailan[1,2], Waleed Aamer[2], Ny Hoang[4], Rulan Shaath[2], Jennifer Howe[3], Olivia Rennie[3], Amira Syed[2], Jyothi Lakshmi[2], Sura Hussein[2], Sujitha Padmajeya[2], Amal Hussein[2], Ilaria Poggiolini[2], Ammira Akil[2], Madeeha Kamal[5], Stephen Scherer[3,4] & Khalid Fakhro[1,2,6#]

[1] Collage of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar, [2] Department of Genetics, Sidra Medicine, Doha, Qatar, [3] The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada, [4] Genetics and Genome Biology Program, The Hospital for Sick Children, Toronto, ON, Canada, [5] Department of Pediatrics, Sidra Medicine, Doha, Qatar, [6] Department of Genetic Medicine, Weill Cornell Medicine, Doha, Qatar

Abstract:
Autism spectrum disorder (ASD) is a neurodevelopmental condition associated with reduced social and communication skills, along with restricted interests and repetitive behaviors. The prevalence of ASD among children in Qatar has been estimated to be 1.14%. Large-scale genomics studies of ASD have identified causative variants that span the entire mutational spectrum, ranging from single nucleotide variants (SNVs) to large structural variants (SVs). In outbred populations, a majority of ASD appears to be caused by *de novo* mutations; however, in consanguineous populations, recessive biallelic variants may contribute to substantial risk of ASD. Whole genome sequencing of 104 families (total samples=402) with children clinically diagnosed with ASD (n=107) was performed at a pediatric tertiary academic center. Using an integrative genetic approach, we examine the genomes for rare SNVs, indels, and SVs, and prioritized deleterious (loss-of-function (LoF) or predicted damaging missense) variants affecting known or candidate ASD genes. In total, we discovered 13 potentially pathogenic SNVs in previously reported ASD genes, including *SCN2A*, *STAG1*, *KDM5B*, and *PTCHD1*. The majority were *de novo* (8/13) or X-linked (3/13), while biallelic variants were present in 2 of the families. In addition, we identified deleterious variants in 22 candidate ASD genes, including 12 *de novo* variants in genes such as *MOV10*, *NPAS3*, *CHD9*, and *HDAC7* and 10 biallelic events in genes such as *TRAPPC9*, *SYNE2*, and *METTL2A*. Moreover, we identified 17 SVs (12 *de novo* and 5 biallelic) affecting ASD candidate genes, including *NIPBL*, *CSNK1A*, *CHRH1*, and *CLN3*. Overall, we identified a putative genetic risk factor in 39.2% (42/107) of ASD cases—29 dominant, 10 recessive, and 3 X-linked. Our study highlights the importance of WGS studies for providing a molecular diagnosis for ASD patients in tertiary care settings and provides a growing resource and first comprehensive look into the genetic structure underlying ASD in the middle east population.

USP37 (Ubiquitin-specific peptidase 37) promotes oncogenesis in osteosarcoma by interacting with PCNA and promoting constitutive replication fork movement

Ravi Chauhan[1], Ashna Gupta[1], Lakshya Malhotra[2], Ajaz A. Bhat[3], Atul Batra[1], Sammer Bakhshi[1], Mehar C Sharma[4], Muzafar Macha[5], Traiq Masoodi[6], Tarang Sharma[1], Mohammad Haris[7], Ethayathulla Abdul Samath[2], Ammira Al-Shabeeb Akil[3], Tej K pandita[8], Mayank Singh[1#]*

[1]Department of Medical Oncology (Lab), Dr. B. R. Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India, [2]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India , [3]Department of Human Genetics, Sidra Medicine, Doha, Qatar, [4]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India, [5]Watson-Crick Centre for Molecular Medicine, Islamic University of Science and Technology, Pulwama, India, [6]Department of Cancer Research, Sidra Medicine, Doha, Qatar, [7]Center for Advanced Metabolic Imaging in Precision Medicine, Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA, [8]Center for Genomics and Precision Medicine, Texas A&M College of Medicine, Houston, TX, USA

Rationale- Osteosarcoma is the heterogeneous primary malignancy of bone. Recent data related to the treatment of osteosarcoma indicates reduced progression free survival (PFS) rates for patients. A better overall understanding of this heterogeneous disease coupled with more effective methods for diagnosis and treatment. Protein de ubiquitination controls many intracellular processes, including cell cycle progression, transcriptional activation, and signal transduction. Ubiquitin-specific peptidases (USPs) remove ubiquitin tags from target proteins to control both protein fate and function. It has emerged as an attractive drug target as it controls myriad aspects of oncogenesis by regulating the stability of oncoproteins. USP37 is one of the least studied members of this family. In a recent study, we have demonstrated that USP37 stabilizes Chk1 (Checkpoint kinase 1), thereby regulating replication stress however our analysis indicted other proteins to be involved in this pehenomenon as well so the current study was an attempt to characterize those using osteosarcoma as a model system.

Methods and Results- TCGA data analysis indicated that overexpression of USP37 correlated with reduced PFS in osteosarcoma patients. Next-generation sequencing (NGS) analysis of osteosarcoma cells indicated that a distinct set of genes were expressed on overexpression or knockdown of USP37. Our data suggest that USP37 overexpression confers survival advantage while its depletion enhances sensitivity in osteosarcoma cell lines U2OS and MG-63 experiencing replication stress. USP37 overexpressing cells were able to resolve DNA damage foci much more effectively than the control cells or cells in which USP37 was depleted in response to genotoxic stress. USP37 depletion results in reduced resolution of γ H2AX and 53BP1 DNA damage foci and an increase in no of collapsed replication fork, indicating the reduced ability of cells to carry out constitutive DNA replication. USP37 was found to interact with PCNA (Proliferative cell nuclear antigen).Interestingly docking studies indicated that USP37 and PCNA interacted with stronger affinity in the presence of double-stranded DNA. We further correlated our data with archived tissue blocks of osteosarcoma patients to see if USP37 levels correlated with patient prognosis and whether its expression correlated with therapeutic intervention. Mechanistically, our data indicated that USP37 is required for tolerance of replication stress and interacted with PCNA, which is required to dock additional replication factors and stabilize the DNA replication fork.

Conclusion-The current data adds a new dimension to the newly explored deubiquitinase USP37 and merits the development of targeting strategies to explore its therapeutic potential in osteosarcoma.

Identification of differentially expressed genes and prognostic biomarkers in colorectal cancer using microarray and RNA-seq data

Tariq Masoodi[1], Ajaz A. Bhat[2], Shilpa Kuttikrishnan[3,4], Gulab Sher[3], Ammira Al-Shabeeb Akil[2], Shahab Uddin[3,4,5]

[1] Cancer Research Department, Sidra Medicine, Doha, Qatar, [2]Department of Human Genetics-Precision Medicine in Diabetes, Obesity and Cancer Program, Sidra Medicine, Doha, Qatar, [3]Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar, [4] College of Pharmacy, Qatar University, Doha, Qatar, [5] Laboratory of Animal Research Center, Qatar University, Doha, Qatar, [6] Dermatology Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar

Abstract

Colorectal cancer (CRC) has become the predominant cancer in recent years, and its incidence and mortality have increased at an alarming rate. Our present study aimed to identify differentially expressed genes (DEGs) between 35 colorectal cancer samples and 24 normal tissues using Gene Expression Omnibus (GEO) dataset GSE23878. Differentially expressed mRNAs were identified using the limma package implemented in the GEO2R analysis tool. Protein-protein interactions (PPI) and gene network clusters were implemented to annotate the likely pathways related to colorectal cancer pathogenesis using STRING, MCODE, and ClueGO plugins in Cytoscape software. Top significant DEGs were validated, and prognostic importance was predicted using TCGA mRNA and clinical data on colorectal cancer. Independent prognostic importance of significant biomarkers was also investigated. At fold change of ±1.5, 1489 significant DEGs were found, including 261 up-regulated and 1228 down-regulated genes. The gene ontology and pathway analysis of DEGs captured several critical biological processes and pathways like cell migration, cell proliferation, immune regulation, immune response, PPAR signaling, ECM-receptor interaction signaling, and calcium signaling pathways. PPI network analysis demonstrated key hub genes linked to CRC pathogenesis, including over-expression of *CXCL8, COL11A1,* and *ADAM12* and down-regulation of *PTPRC, CXCL12, IGF1, FGF2, CLCA4, CLCA1, ANPEP, KLF4, ADH1C,* and *GUCA2A.* All these DEGs were further validated using an additional dataset of 629 colorectal cancer patients from TCGA. Interestingly, CoxPH survival analysis revealed significantly poor overall survival (OS) for *COL11A1*, *ADAM12*, *CLCA4*, *CLCA1*, *ANPEP*, *KLF4*, *ADH1C,* and *GUCA2A.* Multivariate survival analysis found independent prognostic behavior of *COL11A1* and *ADAM12* after adjusting for clinical characteristics, including age, gender, tumor size, lymph node, and distant metastasis. The top significant co-expressed oncogenes for *COL11A1* and *ADAM12* were *PDGFRB, MITF, MAFB, ZNF521*, *MAF*, *CHST11, AKT3, PDCD1LG2*, and *DDR2.* Future studies aim to validate top oncogenes for tumorigenic potential using in vitro and in vivo functional assays.

Pharmacogenomics implementation in cardiovascular disease: lessons learned from a pilot study in the United Arab Emirates

Zeina N. Al-Mahayri[1*], Lubna Q. Khasawneh[1], Mais N. Alqasrawi[1], Sahar Altoum[1], Gohar Jamil[2], Sally Badawi[1], Dana Hamza[1], Lizy George[3], Anwar AlZaabi[2], Husam Ouda[4], Fatima Al-Maskari[5,6], Juma AlKaabi[7], George P. Patrinos[1, 5, 8], Bassam R. Ali[1,5#]

[1]Department of Genetics and Genomics, College of Medicine and Health Sciences, United Arab Emirates University, United Arab Emirates
[2]Department of Medicine, Tawam Hospital, Al-Ain, United Arab Emirates
[3]Nursing Department, Tawam Hospital, Al-Ain, United Arab Emirates
[4]The Heart Medical Center, Al-Ain, United Arab Emirates
[5]Zayed Centre for Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates
[6]Public Health Institute, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates
[7]Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates
[8]School of Health Sciences, Department of Pharmacy, Laboratory of Pharmacogenomics and Individualized Therapy, University of Patras, Patras, Greece

***Presenting Author**
#Corresponding Author

Pharmacogenomic (PGx) testing has proved its utility and cost-effectiveness for some common cardiovascular disease (CVD) medications. PGx-guided dosing guidelines are available for multiple CVD drugs, including clopidogrel, warfarin, and statins. The United Arab Emirates (UAE) population is diverse and multiethnic. PGx testing is not part of the standard of care in most global healthcare settings, including the UAE. The first pharmacogenomic implementation clinical study in CVD has been approved recently, but multiple considerations needed evaluation before commencing. The current report appraises the PGx-clinical implementation procedure and the potential benefits of pursuing PGx-implementation initiatives in the UAE with global implications. For the current pilot study, 160 patients prescribed one or more of the following drugs: clopidogrel, atorvastatin, rosuvastatin, and warfarin, were recruited. Genotyping selected genetic variants at genes interacting with the study drugs was performed by real-time PCR. The genotypes and inferred haplotypes, diplotypes, and predicted phenotypes revealed that 11.9% of the participants were poor CYP2C19 metabolizers, 35% intermediate metabolizers, 28.1% normal metabolizers, and 25% rapid or ultrarapid metabolizers. Notably, 46.9% of our cohort should receive a recommendation to avoid using clopidogrel or to consider an alternative medication. Regarding warfarin, only 20% of the participants exhibited reference alleles at *VKORC1*-1639G>A, *CYP2C9**2, and *CYP2C9**3, leaving 80% with alternative genotypes at any of the two genes that can be integrated into the warfarin dosing algorithms and can be used whenever the patient receives a warfarin prescription. For statins, 31.5% of patients carried at least one allele at the genotyped *SLCO1B1* variant (rs4149056), increasing their risk of developing myopathy. 96% of our cohort received at least one PGx-generated clinical recommendation for the studied drugs.
The current pilot analysis verified the feasibility of PGx testing and the unforeseen high frequencies of patients currently treated with suboptimal drug regimens, which may potentially benefit from PGx testing.

Cancer stem cells targeting; the lessons from the interaction of the immune system, the cancer stem cells and the tumor niche

Hajar Rajayi[1], Parsova Tavasolian[1*], Alaleh Rezalotfi[1], Marzieh Ebrahimi[1,2#]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Failure of treatment strategies against cancers is a major issue engaging many scientists to investigate the possible resistance factors. Cancer stem cells (CSCs) subvert promising therapeutic methods by developing resistant cancers. These pluripotent cells are located in individual microenvironments called cancer niche. CSCs affect the immune cells and on the flip side, the immune cells in the cancer niche influence them. Thereby, the interaction between CSCs and immune cells in cancer niche needs to be clearly studied in order to develop novel efficient methods of immune-based cancer treatment. In this article, we review literature about the suggested methods of CSC escape from immune responses and the effect of cancer niche characteristics on the ability of CSCs to develop resistant strains of cancers. Moreover, we discuss immune-mediated tumor targeting methods and bring in trials focused on CSC targeted therapies. We aim to help physicians to reach a consensus about using CSC-targeted immunotherapy methods and emerge novel immunotherapy methods through disrupting the interaction between immune cells and CSCs in the tumor microenvironment.

Keywords: Cancer stem cell, immune system, immune targeting, tumor niche

VWA8 causes developmental delay, microcephaly, scoliosis and responsible for skeletal development and morphogenesis in Zebrafish

Muhammad Umair[1*], Muhammad Farooq Khan[2], Abeer Al Tuwaijri[1], Majid Alfadhel[1,3#]

[1]Medical Genomics Research Department, King Abdullah International Medical Research Center (KAIMRC), King Saud Bin Abdulaziz University for Health Sciences, Ministry of National Guard Health Affairs (MNGH), Riyadh, Saudi Arabia.

[2]Bioproducts Research Chair, College of Science, Department of Zoology, King Saud University, P.O Box 2455 Riyadh 11451, Kingdom of Saudi Arabia

[3]Genetics and Precision Medicine department (GPM), King Abdullah Specialized Children's Hospital (KASCH), King Abdulaziz Medical City, Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia.

Theme: Animal, cellular, and organoid models for human disease modeling

ABSTRACT

Von Willebrand A Domain-Containing Protein 8 (VWA8), is a poorly characterized, mitochondrial matrix-targeted protein having a putative ATPase activity.VWA8 is consist of ATPase-domains and a VWFA domain with ATPase activity.

In the present study, we describe an extended Saudi family having nine affected individuals segregating a complex developmental syndrome in an autosomal recessive fashion. DNA from the affected individuals was subjected to whole-exome sequencing (WES) followed by standard Sanger sequencing.

WES revealed a homozygous missense variant [c.947A>G; p.(Asp316Gly)] in exon 8 of the *VWA8* gene, which perfectly segregated with the disease phenotype. Furthermore, VWA8 knockout zebrafish morpholinos were used to study the phenotypic effect of *VWA8* gene on zebrafish development. Using zebrafish morpholino, we observed delayed development at an early stage, lack of movement, light sensitivity, severe skeletal deformity, scoliosis, and facial dysmorphism. 3D protein modeling revealed substantial structural changes in the secondary structure of the mutated VWA8 protein.

We reported the first homozygous variant identified in the *VWA8* gene associated with global developmental delay, microcephaly, scoliosis, limbs, and cardiovascular malformations in humans. We provide genetic and molecular evidence using zebrafish morpholino that associated bi-allelic variant in the *VWA8* gene with a complex developmental syndrome in humans.

The genetic burden of familial hypercholesterolemia in the Qatari population

[*]Geethanjali Devadoss Gandhi [a,b], Waleed Aamer [b], Navaneethakrishnan Krishnamoorthy [b], Najeeb Syed [c], Elbay Aliyev [b], Aljazi Al-Maraghi [b], Mohammed Kohailan[a,b], Mohammed Elanbari[d], Qatar Genome Program Research Consortium (QGPRC) [†], Borbala Mifsud [a], Younes Mokrab[a,e,f], Charbel Abi Khalil [f,g], and [#]Khalid A. Fakhro [a,b,f]

[a] College of Health and Life Sciences, Hamad Bin Khalifa University (HBKU), Doha, Qatar

[b] Human Genetics Department, Sidra Medicine, Doha, Qatar

[c] Bioinformatics, Genomic Data Science Core Sec, Sidra Medicine, Doha, Qatar

[d] Clinical Research Centre, Sidra Medicine, Doha, Qatar

[e] Laboratory of Medical and Population Genomics, Sidra Medicine, Doha, Qatar

[f] Department of Genetic Medicine, Weill Cornell Medicine, Qatar (WCM-Q)

[g] Joan and Sanford I. Weill Department of Medicine. Weill Cornell Medicine, New York, US

Background: The genetic architecture underlying Familial Hypercholesterolemia (FH) in the Middle East region is yet to be fully described.

Methods: We present a large-scale characterization of genetic alleles in known FH-causing genes using a whole-genome sequencing (WGS) dataset of 6,140 adult Qatari subjects from the Qatar Genome Program (QGP), for whom an FH diagnosis was established using Dutch Lipid Clinic Network (DLCN) criteria.

Results and conclusion: Using DLCN criteria, we identified eight (0.1%) 'definite', 41 (0.7%) 'probable' and 332 (5.4%) 'possible' FH individuals. We examined known and potentially novel pathogenic variants in three major FH genes, *LDLR*, *APOB*, and *PCSK9*, as well as recessive variants in *LDLRAP1*, *ABCG5*, *ABCG8*, and *LIPA*. We identified four known pathogenic single-nucleotide variants (SNVs) in the *LDLR* gene present in 11 heterozygous individuals and thus estimated a prevalence of known FH-causing variants of 1:558 in Qatar. We found 14 putatively novel SNVs with potential pathogenicity (estimated clinical penetrance range: 14%–100%) and one rare copy number variant in *PCSK9*. We observed homozygous individuals carrying pathogenic variants for recessive genes such as *LDLRAP1* and *ABCG8*. Further, we explored the utility of globally established 12 SNP LDL-C SNP score to predict polygenic FH risk in Arab populations. We found that 90% of 'definite or probable' FH individuals who do not carry mutations in the three major FH genes had LDL-C SNP scores in the top three quartiles of the 'unlikely' FH group, suggesting a polygenic inheritance.

The Potential impact of *Haemophilus* In Obese Population With Acute Graft-versus-Host Disease (aGvHD)

Arun Prasath Lakshmanan[1,*], Sara Deola[1], Lupsa Nikolett[2], Zoltan Pas[2], Annalisa Terranegra[1,#]

[1]Research Department, Sidra Medicine, P.O. Box 26999, Doha, Qatar.
[2]Department of Genetics, Cell and Immunobiology, Semmelweis University, 1089 Budapest, Hungary.

[*]Presenting author: alakshmanan@sidra.org
[#]Corresponding author: aterranegra@sidra.org

Abstract:

Background/Objective: Acute graft-versus-host disease (aGvHD) occurs after an allogeneic hematopoietic stem cell transplant (aHSCT), and despite significant advancements in the treatment strategy, still it remains a life-threatening complication. This is due to the reaction of donor immune cells (activated T cells) with host tissues or organs. Recently, dysbiosis in the gut microbiota composition has been implicated in the pathogenesis of aGvHD condition. In this study, we aimed to explore any early microbial biomarker of aGVHD and investigate whether body mass index (BMI) plays a crucial role in the pathogenesis of aGvHD through the modulation of gut microbiota.
Methods: Adult patients receiving aHSCT for various indications were recruited for the study at the Department of Hematology and Stem Cell Transplantation, South Pest Central Hospital—National Institute for Hematology and Infectious Diseases, Budapest, Hungary. Anthropometric data (BMI kg/m2) were collected before the transplant. The stool samples were collected at various time points (TPs; Day 0, 7, 14, 21, 28, 100+). The DNA was extracted from the stool samples using a Qiagen Fast Stool Mini kit, and the extracted DNA was amplified using a primer that targets the V3-V4 region. Then, 16s rDNA sequencing was performed on Illumina Miseq (600 cycles) kit. The raw data was processed for taxonomic classification using the QIIME v1.9.0 pipeline. Further downstream analyses, such as diversity indices, microbial markers (LEfSe), and predicted functional pathway (PICRUSt) analysis were performed.
Results: On day 14, we observed reduced microbial diversity in the GvHD and non-GvHD groups. We found the genera *Faecalibacterium*, and *Haemophilus* increased in aGvHD, and *Sutterella* increased in the non-GvHD group. Moreover, on day 14, higher levels of pyruvate metabolism and fatty acid synthesis were observed in the obese aGvHD group, whereas galactose metabolism was higher in the healthy weight aGvHD group.
Conclusion: These results suggest that the genera *Faecalibacterium*, *Haemophilus*, and *Sutterella* can be targeted as early biomarkers of aGVHD about 2 weeks after transplant. Moreover, the obesity condition might be detrimental to aGvHD possibly through the increased abundance of the genus *Haemophilus* which in turn potentiates the pyruvate metabolism and fatty acid synthesis.

FTO expression is associated with poor prognosis in colorectal cancer and its inhibition in human colorectal cells enhances sensitivity to chemotherapeutic drugs

[1]Hana Q. Sadida, [1]Sara K. Al-Marzooqi, [1]Fajr Al-Marzooqi, [1]Sabah Nisar, [1]Summaiya Khatoon, [2]Tariq Masoodi, [1]Ammira Al-Shabeeb Akil, [1]Ajaz A. Bhat

[1]Department of Human Genetics, Sidra Medicine, Doha, Qatar, [2]Department of Cancer Research, Sidra Medicine, Doha, Qatar,

Abstract

Colorectal cancer (CRC) is the third leading cause of cancer-related mortality worldwide. Conventional treatments for CRC include surgery, radiotherapy, and chemotherapy. Despite these advances, CRC remains a leading cause of cancer-related deaths due to chemoresistance, different toxicities, and undesirable side effects exhibited by chemotherapeutic drugs. Recently, fat mass and obesity-associated protein (FTO) has been implicated in obesity and cancer. The FTO protein is well known for its strong association with obesity, fat mass regulation, and adipogenesis. In Qatar, approximately 70% of the adult Qatari population is overweight, while 41% are obese. As obesity is considered one of the risk factors for certain cancers, FTO has been an essential link between obesity and tumorigenesis. The oncogenic role of FTO has been demonstrated in cancers such as acute myeloid leukemia and glioblastoma, and the N^6-methyladenosine (m^6A) demethylase activity exhibited by FTO is found to be linked to its role in adipogenesis and tumorigenesis. The m^6A modification controls the stability of messenger RNA (mRNA) and functions as a posttranscriptional modulator of gene expression. Based on the significant role of FTO in tumorigenesis, there is a strong possibility that FTO could serve as an essential therapeutic target in the future. Using a large CRC patient database, we found FTO expression significantly upregulated in cancer samples. High FTO expression was associated with poor overall survival and microsatellite stable (MSS) tumors. Furthermore, FTO expression negatively correlates with $CD4^+$ T cells, Th2 cells, Th17 cells, and dendritic cells and positively with TGF-β response and macrophages. Using in vitro cell viability assay, we found that FTO inhibition in two human colorectal cancer cell lines (SW480/SW620) increases the sensitivity to cisplatin, the mainstay of standard therapy in colon cancer. Future studies are aimed at delineating the mechanism of chemosensitivity to FTO inhibition. Our study will be a great addition to FTO research in the field of obesity-cancer links and will serve as a platform for extensive research towards developing potential diagnostic and/or prognostic biomarkers.

p53 inhibition in pancreatic progenitors enhances the differentiation of human pluripotent stem cells into pancreatic β-cells

Idil I. Aigha[1] and Essam M. Abdelalim[1,2 *]

[1]*College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar Foundation.*
[2]*Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation.*

Abstract

Background and aim: The multipotent pancreatic progenitor cells (MPCs) co-expressing the transcription factors (TFs), PDX1 and NKX6.1, are the source of functional insulin-secreting β-cells. Therefore, enhancing the generation of PDX1+/NKX6.1+ MPCs can improve the β-cell differentiation. The aim of this study was to examine the effect of p53 inhibition in MPCs on the generation of PDX1+/NKX6+ MPCs that can generate functional pancreatic β-cells.
Materials and Methods: Human pluripotent stem cells (hPSCs) were differentiated into MPCs and β-cells. hPSC-MPCs (stage 4) were treated with different concentrations of p53 inhibitors, and their effect was evaluated using different approaches. The NKX6.1 was overexpressed at the MPCs.
Results: Chemical inhibition of p53 (Pifithrin-μ) at the MPC stage resulted in a significant increase in the number of PDX1+/NKX6.1+ cells and a reduction in the number of CHGA+/NKX6.1- cells. Further differentiation of MPCs treated with Pifithrin-μ into pancreatic β-cells showed that Pifithrin-μ treatment did not significantly change the number of C-Peptide+ cells; however, the number of cells co-expressing C-Peptide and Glucagon (polyhormonal) was significantly reduced in the pifithrin-μ treated cells compared to controls. Interestingly, overexpression of NKX6.1 in hPSC-MPCs enhanced the expression of key MPC genes and dramatically suppressed p53 expression.
Conclusion: Our findings demonstrated that the inhibition of p53 during stage 4 of differentiation enhanced MPC generation, prevented premature endocrine induction and favored the differentiation into monohormonal β-cells. These findings suggest that adding a

p53 inhibitor to the differentiation media can significantly enhance the generation of monohormonal β-cells.

The QGPRS Study: Development of optimized Polygenic Risk Score models for predicting diabetic complications in Qatar

*Ikhlak Ahmed[1], Shahrad Taheri[2,] Jyothi Lakshmi Janardhanan[1], Khalid Fakhro[1,3,4,5], *Ammira Al-Shabeeb Akil[1,3]*

1. Precision Medicine of Diabetes, Obesity and Cancer Research group, Precision Medicine Program, Department of Human Genetics, Sidra Medicine, P.O. Box 26999, Doha, Qatar
2. Qatar Metabolic Institute, Hamad Medical Corporation, Hamad Bin Khalifa Medical City, Doha, Qatar
3. Laboratory of Genomic Medicine-Precision Medicine Program, Department of Human Genetics Sidra Medicine, P.O. Box 26999, Doha, Qatar
4. College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, P.O. Box 34110, Qatar
5. Department of Genetic Medicine, Weill Cornell Medical College, Doha, P.O. Box 24144, Qatar
* This work is supported by the PPM award (PPM 03-0311-190017) from QNRF to Dr. Ammira Al-Shabeeb Akil

Background: Type 2 diabetes (T2D) is a common disease with multiple genome-wide association studies having reported >500 genetic variants associated with the disease. Translating these associations into specific predictive models has remained a challenge to predict and prevent disease risk. Polygenic scores (PRS) have been shown to provide enhanced power for detecting disease susceptibility and stratifying the high-risk individuals. PRS is an individual-level score that encapsulates the impact of multiple genetic variations on an individual's phenotype. Therefore, the score can be utilized as an outcome predictor in clinical prediction and disease screening programs as it provides an assessment of the entire genetic risk of an individual for a particular trait. The score is generated as a function of the total number of risk variants a person carries weighted by the effect size of each of the individual allele on the phenotype. An independent large-scaled genome-wide association study (GWAS) is required as a discovery cohort to estimate the effect size of each of the genetic variants on the phenotype or disease.
Objective: To identify a set of genomic variants that are associated with diabetes risk in Middle Eastern populations. Construct PRS models using the identified risk variants to evaluate their predictive performance for the disease.
Methods: We carried out multiple genome-wide association studies (GWASes) to identify genomic variants linked to clinical characteristics of diabetes. A local cohort of Qatar Genome Programme participants sorted into cases and controls served as the basis for each GWAS, which was used to find a discovery set of variants. For an optimum predictive performance, multiple weighted PRS models were constructed using the identified discovery set of variants.
Results: Our PRS models displayed a strong ability to distinguish cases from controls, and the receiver operating characteristic (ROC) curves demonstrate that weighted models have a better predictive performance than the original PRS model.
Conclusion: PRS models have the potential to identify individuals that are at-risk for developing T2D and associated complications in our population. Stratifying people with T2D into groups based on their predisposition for problems could help identify those who would benefit most from an early intervention.

Functional Genomics studies on variants associated with childhood obesity: preliminary results from the IVORY project

*Ilaria Poggiolini[1,2], Ikhlak Ahmed[1], Saadya Amrullah[1], Munazza Tamkeen[1], Alanoud Abdulla[1], Amal Hussein[1,2], Reem Al-Karbi[1], Khalid Fakhro[1,2,3,4], *Ammira Al-Shabeeb Akil[1,2]*

1. Precision Medicine of Diabetes, Obesity and Cancer Research group, Precision Medicine Program, Department of Human Genetics, Sidra Medicine, P.O. Box 26999, Doha, Qatar
2. Laboratory of genomic medicine-Precision Medicine Program, Department of Human Genetics Sidra Medicine, P.O. Box 26999, Doha, Qatar
3. College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, P.O. Box 34110, Qatar
4. Department of Genetic Medicine, Weill Cornell Medical College, Doha, P.O. Box 24144, Qatar

Background: Obesity accounts for a growing burden of disease globally. Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016 (World Health Organization). A number of genes including, *LEP*, *LEPR*, *POMC*, *MC4R*, *PPCSK1*, *PHIP*, *DGKI*, *ZMYM4*, *CALCR*, *GIPR*, *GPR151* and *GPR75* have been implicated in the risk of becoming obese.

Objective: To identify potential variants of interest in various genes linked to obesity.

Methods: The IVORY (**I**nvestigating molecular pathways compelling novel **V**ariants in major human **O**besity **R**elated genes: targeted and effective drug deliver**Y** Approach) project aims to recruit 150-200 obese 6-18 years old children from the Obesity Clinic and General pediatrics at Sidra Medicine to identify obesity-associated genetic variants. Functional validation using cellular models will be performed to analyze the effect of potential disease-causing variants on cellular pathways including regulation of energy homeostasis and transport functions.

Results: To date, our preliminary results show that after transfection of HEK293T cells with *GPR75* wild-type and two rare, predicted loss-of-function variants (*GPR75* MUT1 and *GPR75* MUT2), the exogenous proteins were found both in the cytosolic and in the membrane fractions. The *GPR75* MUT2 variant was the most efficiently expressed, which could relate to protein stability since the mRNA level of this variant was the same as the wild-type and *GPR75* MUT1 mRNAs. Moreover, after EGF stimulation we observed that ERK and AKT phosphorylation was higher in cells transfected with wild-type GRP75 compared to mock-transfected cells and mutant GRP75-expressing cells.

Conclusions: Functional genomic studies on variants associated with obesity in children have the potential for targeted and personalized medicine for children suffering from obesity and the associated comorbidities. The preliminary data suggest that the *GRP75* variants may display loss of function which warrants further investigation, such as effects of these variants on energy homeostasis including glucose and lactate metabolism.

Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats

Dariush Haghmorad[1,2], Mohammad Bagher Mahmoudi[3], Pardis Haghighi[4], Paria Alidadiani [4] Ensieh Shahvazian [4,] Parsova Tavasolian[4*], Mahmoud Hosseini[5], Mahmoud Mahmoudi[4#]

1. Department of Pathology and Laboratory Medicine, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran;
2. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran;
3. Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;
4. Immunology Research Center, BuAli Research Institute, Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences; Iran
5. Neuroscience Research Center, Department of Physiology, School of Medicine, Mashhad University of Medical Sciences Iran

Anacyclus Pyrethrum (AP) and Tribulus Terrestris (TT) have been reported as male infertility treatment in several studies; however, in Iranian traditional medicine these two plants are prescribed simultaneously. In this study, we aimed to determine the effects of AP and TT extracts both separately and simultaneously on the male Wistar rat fertility parameters. 32 male Wistar rats were divided into 4 groups: Control, TT, AP, and AT treated groups. Treatment continued for 25 days and rats were weighed daily. Their testes were dissected for histological studies. Sperm analysis including sperm count, viability and motility were performed. Serum was obtained to evaluate testosterone, LH and FSH levels. Histological studies were conducted to study Leydig, and Sertoli cells, spermatogonia and spermatid cell numbers, and to measure seminiferous diameter and epithelium thickness. Sperm count increased in all the treatment groups. Sperm viability and motility in AT and AP groups were elevated. TT and AT groups showed significantly increased testosterone level compared to control group (P=004, P=0.000, respectively) and TT, AP and AT treatment groups showed increased LH level (P=0.002, P=0.03 and P=0.000, respectively) compared to control, while only AT group showed increased FSH (p=0.006) compared to control. Histological studies showed significant increase of spermatogonia, Leydig and Sertoli cell numbers and epithelial thickness in AT group compared to other groups. All the treatment groups had higher number of Leydig, spermatogonia and spermatid cells. TT and AP improved sexual parameters; however, their simultaneous administration had higher improving effects on studied parameters.

Keywords: Tribulus; Testosterone; Receptors, FSH; Receptors, LH

Patterns and distribution of de novo mutations in multiplex Middle Eastern families

Muhammad Kohailan[a,b,*], Waleed Aamer[a], Najeeb Syed[c], Sujitha Padmajeya[a], Sura Hussein[a], Amira Sayed[a], Jyothi Janardhanan[a], Sasirekha Palaniswamy[a], Nady El hajj[b], Ammira Al-Shabeeb Akil[a] and Khalid Fakhro[a,b,#]

[a] Department of Human Genetics, Sidra Medicine, Doha P.O. Box 26999, Qatar.

[b] College of Health and Life Sciences, Hamad Bin Khalifa University, Doha P.O. Box 34110, Qatar.

[c] Biomedical Informatics Division, Sidra Medicine, Doha P.O. Box 26999, Qatar.

While *de novo* mutations (DNMs) are a critical driver of diversity in any species, they also play a major role in severe genetic disorders. Thus, it is important to understand the rate, distribution and pattern of DNMs. Considering their rarity in the genome, detecting such mutations requires prudent approach that can extract them from NGS data. Here, we applied a combinatorial approach by using three different tools to generate an integrated list of DNMs per individual.

For 353 trios, we identified 24,808 de novo single-nucleotide variants (SNVs) and 2,405 insertions-deletions (INDELs), with a median of 70 SNVs and 6 INDELs per individual. We calculated a median de novo mutation rate of 1.25×10^{-8} and 1.07×10^{-9} per base per generation for SNVs and INDELs, respectively.

We determined the parent-of-origin for around 13% of the de novo variants, and found a paternal to maternal DNMs ratio of approximately 3.96:1. We then plotted the number of phased DNMs in each individual against parental age at conception, and observed a significant increase of 1.79 DNMS per year of paternal age and 0.34 DNMS per year of Maternal age. However, this correlation substantially differs between families.

We also examined the DNM spectra and mutational signature. We found a clear enrichment of transitions over transversions and that the mutations at CpG sites contribute to a large fraction of DNM events. We then compared the mutation rates at CpGs with respect to the

level of methylation, and found that highly methylated CpGs are 2.05 times more likely to have mutations than low-methylation sites.

We found no significant DNM count differences in terms of consanguinity, ethnicity or disease phenotype. However, Middle-Eastern families with Arab ancestry seem to have fewer DNMs than African families. Therefore, larger study cohorts are required to investigate this correlation.

Discovery of a novel translational checkpoint during cell cycle progress

Mahmoud Izadi [a*], Tayyiba Akbar Ali [a], Farah M. Shurrab [a], Ehsan Pourkarimi [a#]

[a] Division of Genomics and Translational Medicine, College of Health and Life Sciences, Hamad Bin Khalifa University, Doha 34110, Qatar.

[*]Presenting Author: Ph.D. student in Genomics and Precision Medicine (GPM), College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation, maiz30979@hbku.edu.qa

[#]Corresponding author: Assistant Professor, Division of Genomics and Translational Medicine (GTM), College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation, edaryakenari@hbku.edu.qa

Abstract:

Aminoacyl-tRNA synthetases (ARSs) are a family of enzymes responsible for the high-fidelity loading of amino acids to their cognate transfer RNAs (tRNAs). Mutations in ARSs have been observed in several human diseases, including cancer, neuropathy, and diabetes. Among them, mutations in Tryptophanyl-tRNA synthetase (WARS) have been reported in different cancers. A significant body of research indicates that cell cycle proteins regulate translation; however, the translation regulating the cell cycle proteins has not been revealed yet. Various cell cycle-related protein kinases and phosphatases control cell cycle progression and cell division to maintain the integrity of the inherited genome. Cell cycle regulation is mainly governed by cyclins and cyclin-dependent kinases (CDKs). In tumor cells, dysregulation of CDKs activity leads to uncontrolled proliferation and subsequent tumor formation. *Caenorhabditis elegans* (*C. elegans*) germline is the only mitotically active region in the adult worms, which makes them the ideal model organism to study cell cycle and division. Previously, we reported that *wars-1* depletion is associated with defects in germ cell development and cell division in *C. elegans*. Using cytological markers, fluorescent microscopy, and a reverse genetics approach, we found that knocking down (KD) *wars-1* using RNA interference (RNAi) resulted in the inactivation of CDK-1, the canonical marker of cell cycle arrest, in the mitotic cells of the *C. elegans* germline. The reduced number of cells at the M phase of mitosis upon *wars-1* KD indicates cells are arrested at the G2/M phase. Therefore, we suggest a novel translational checkpoint at the G2/M phase that links translation status to the cell cycle regulator, a potential target for personalized cancer therapy. This signifies using CDK1 inhibitors currently undergoing clinical trials, such as flavopiridol, dinaciclib, and Milciclib, in patients carrying an oncogenic mutation in WARS1 to taper down CDK1 hyperactivity and ultimately prevent tumor cell proliferation.

Mapping the road of GVHD with a transcriptome "compass". A new approach to solve post transplantation dilemmas

Merav Bar[1], Darawan Rinchai[2†], Mohammed El Anbari[2†], Mohammed Toufiq[2], Dhanya Kizhakayil[2], Harshitha S. Manjunath[2], Rebecca Mathew[2], Irene Cavattoni[3], Sabine Forer[3], Hani Bibawi[4], Ahmad Alater[2], Clarisa Brown[1], Nancy Miles[1], Phuong Vo[1], Davide Bedognetti[2], Sara Tomei[2], Ayman Saleh[5], Chiara Cugno[2,5], Damien Chaussabel[2], **Sara Deola[2#]**

[†]These authors have contributed equally to this work

[#]**Corresponding author, presenting author**

1 Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

2 Research Department, Sidra Medicine, Doha, Qatar

3 Hematology and BMT, General Hospital, Bolzano, Italy

4 Department of Pathology and Laboratory Medicine Sidra Medicine, Sidra Medicine, Doha, Qatar

5 Hematology, Oncology, BMT Pediatric Hematology/Oncology Division Sidra Medicine Doha, Qatar

Abstract

Graft versus host disease (GVHD) and graft versus tumor (GVT) effect after allogeneic hematopoietic cell transplantation (allo-HCT) result from complex interactions between the donor immune system and the recipient environment, and may be influenced by external variables, such as infections or drugs. High-temporal longitudinal monitoring would be key to identify many still unknown triggering events of GVHD and GVT and to intercept these events before their occurrence.

In this study, we implemented a targeted multiplex microfluidics q-PCR based transcriptional fingerprint assay (TFA) on 50ul of blood collected by a simple fingerstick to evaluate post-allo-HCT systemic immune perturbations associated with the development of GVHD.

Fluctuations of a panel of 264 genes were measured in allo-HCT patients by analysis of 50ul serial blood samples. Thirty-one blood samples were collected pre-transplantation and 245 samples were collected weekly or biweekly post-transplant at a median of 215 days post-transplantation and correlated with detailed clinical annotations. Cross-sectional and longitudinal analyses were performed.

Signatures of neutrophil activation and interferon (IFN) characterized the onset of acute GVHD, while protein synthesis, inflammation, neutrophil/neutrophil activation, interferon and erythroid pathways distinguished the course of GVHD after allo-HSCT. Most of these signatures displayed high-level fluctuations, and some of them were associated with GVHD progression.

Notably, different forms of GVHD displayed distinct signatures. While an ongoing cytotoxic response was modulated in chronic mild GVHD, protein-synthesis and B-cell related signatures characterized late acute/overlap GVHD, and un unexpected erythroid signature associated with immune suppressive properties distinguished patients with acute- and mild chronic GVHD.

Our micro-invasive approach unveiled the molecular heterogeneity of GVHD and identified hierarchically important biological processes conducive to different forms of GVHD. These findings increase our understanding of GVHD and reveal potentially targetable alterations.

This approach might be implemented clinically to intercept GVHD before its occurrence and to modulate therapeutic interventions accordingly.

Mapping the road of GVHD with a transcriptome "compass". A new approach to solve post transplantation dilemmas

Fatemeh Nasrabadi[a*], Sara Chitgaran[a] , Moein Farshchian[a] , Vesal Yaghoobi[b,c] #

[a] Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture, and Research (ACECR), Mashhad, Iran.
[b] Department of Pathology, Yale School of Medicine, New Haven, CT, USA.
[c] Department of Pathology, Tufts Medical Center, Boston, MA, USA.

#vesal.yaghoobi@yale.edu

Ductal Carcinoma in Situ (DCIS) is a premalignant lesion of breast cancer; However, many of breast cancer cases show no associated DCIS. Instead, we noted some poorly characterized morphologic features, that we believe represent an alternative mechanism of carcinogenesis. Here we show preliminary evidence supporting a novel and unique model for tumorigenesis in breast cancer which is different from the current accepted model.

In order to prove the neoplasticism nature of the mentioned structures, we studied transcriptome profiling using GeoMx Digital Spatial Profiler. By taking advantage of the GeoMx® Cancer Transcriptome Atlas (CTA), the expression of over 1800 genes with spatial resolution from distinct regions of interest were measured in 4 Tissue Microarrays (TMAs) containing several selected breast tumor and normal breast tissue specimens acquired through mastectomy and reduction mammoplasty procedures, respectively, at Yale New Haven Hospital between the years 2011-2012.

Our results showed activation of tumorigenesis pathways related in early stages of breast cancer and may have significant implications for choosing therapeutics-diagnosis biomarkers. These data led us to propose a new tumorigenesis model in which instead of filling the breast ducts lumen as happens in DCIS, cells bud off from the basal layer of breast duct without any breakage in this layer. These budding cells, which come off the ducts, disseminate and seed the invasive tumor, without apparent microinvasion seen as the breakthrough event in traditional DCIS.

Metabolomics profiling upranks the role of natural steroids and phenylalanine metabolism over oxidative stress in insulin resistance amongst lean subjects.

Ilhame Diboun[1]*, Layla Al-Mansoori[2], Omar Albagha[3], Khalid Fakhro[3,4,5], Younes Mokrab[1,4], Mohamed A Elrayess[2#]

1 Medical and Population Genetics Lab, Human Genetics Department, Research Division, Sidra Medicine, Doha, Qatar
2 Biomedical Research Center (BRC), Qatar University, Doha, Qatar
3 College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar
4 Department of Genetic Medicine, Weill Cornell Medicine, Qatar
5 Department of Human Genetics, Sidra Medicine, Doha, Qatar

Insulin resistance (IR) is a prediabetic condition characterized by a loss of normal insulin sensitivity (IS). IR is strongly associated with obesity, but it is also occasionally observed amongst lean individuals. This study aims to characterize the metabolic correlates of IR in healthy lean to shed light on the underlying mechanisms.

In this cross-sectional study, clinical and metabolic data were obtained for 200 lean healthy females (100 IR and 100 IS) from Qatar Biobank and another set of 107 obese subjects, (41 IR and 20 IS) from local hospitals in Qatar. Linear models comparing IR versus IS revealed upregulation of anabolic steroids, for both lean and obese including Androsterone Glucoronide and Epiandrosterone, in addition to derivatives of Phenylalanine including the microbiota-product 1-Carboxyethylphenylalanine. Conversely, interaction analysis looking at differential IR/IS effects between obese and lean revealed that levels of long chain unsaturated fatty acids and markers of oxidative stress, including 2-Hydroxybutyrate, were uniquely elevated amongst obese IR but not lean IR, in comparison to their respective baseline levels in IS. Elastic-Net-Regularized-Generalized-Linear-Model was used to select a subset of 20 best predictor metabolites of IR in the lean and ROC analysis suggested a high discriminant capacity (AUC=0.93). Amongst the predictor set of metabolites selected were Phenylalanine derivatives, steroids, xenobiotics (PFOS and Piperidinone), Glucose and metabolic traces of Lysine metabolism.

Our data suggest an interplay of potentially genetic and environmental factors underlying IR in the lean population as evident in the enrichment in natural steroids but also food-derived amino acids including Lysine and Phenylalanine. Importantly, our study suggests a different profile of risk factors to IR in the lean in comparison to obese subjects where IR is thought to be primarily associated with oxidative stress. Our subset of predictive IR metabolites in lean subjects may hold clinical value, hence its validation is warranted.

DANNA Protocol: Deep Analysis of Novel Regulatory Dual Expresser Lymphocytes in New-onset Autoimmune Type-1 Diabetes

Mubarak Ziab[1], Muzammil Ahmad Khan[1], Jean-Charles Grivel[2], Goran Petrovski3, Igor Pavlovski[2], Selma Maacha[2], Sura Ahmed Hussein[1], Jyothi Lakshmi Janardhanan[1], Amira Sayed[1], Hajar Dauleh[3], Ahmed Elawwa[3], Tasneem Riyad Abdel Karim[3], Mohammad Bashir[4], Khalid Fakhro[1], Ammira Alshabeeb Akil[1#]

[1] Precision Medicine of Diabetes, Obesity, and Cancer, Precision Medicine Program, Department of Human Genetics, Sidra Medicine, P.O. Box 26999, Doha, Qatar; [2] Deep Phenotyping Core, Sidra Medicine, P.O. Box 26999, Doha, Qatar; [3] Department of Pediatric Medicine, Division of Endocrinology and Diabetes, Sidra Medicine, P.O. Box 26999, Doha, Qatar; [4] Department of Endocrinology, Qatar Metabolic Institute, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar

Introduction: Type 1 diabetes (T1D) is a chronic autoimmune disease resulting from the destruction of pancreatic β-islets. A novel type of lymphocytes expressing common lineage markers of conventional T-lymphocytes and B-lymphocytes, termed dual expressers (DE), has been recently identified and shown to be over-expressed in T1D patients compared to healthy individuals. Further, the DEs contributed to autoimmunity through a conserved autoantigen (x-idiotype, "x-Id") encoded within the CDR3 region of the heavy chain of the B-cell receptor (BCR). This potent neoantigen has an optimal binding affinity to the diabetogenic HLA-DQ8 molecules, which might stimulate the autoreactive $CD4^+$ T-cells. Due to its conserved sequence and expression, identifying the x-Id could serve as a universal biomarker for targeted therapies in T1D.

Study objectives: This study aims to identify the DE lymphocytes in patients from the local T1D biorepository and explore their role in autoimmunity.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from T1D patients and healthy controls and stained using fluorophore tagged antibodies against T-cell receptor (TCRαβ), IgD, CD5, and CD19 markers (Ahmed et al., 2019). Fluorescence-activated cell sorting was then used to immunophenotype single $TCR\alpha\beta^+IgD^+CD5^+CD19^+$ DE-cells. Screening of the x-Id was performed through RT-PCR as previously described [1].

Results: Despite the small number of clinical samples currently processed, our preliminary immunophenotyping findings revealed relatively high expression of DEs in T1D patients (mean normalized frequency = 0.15%, n = 2) compared to healthy controls (0.04%, n = 2). However, our RT-PCRs did not identify the previously reported x-Id autoantigen. We are currently replicating these findings in a larger number of patients.

Conclusions: T1D patients of the local population might exhibit the previously described DE-lymphocytes which could contribute to autoimmunity. However, the uniqueness of this population might also lead to discovering key drivers of the autoimmune process of T1D and other autoimmune diseases.

1. Ahmed, R*., et al., A Public BCR Present in a Unique Dual-Receptor-Expressing Lymphocyte from Type 1 Diabetes Patients Encodes a Potent T Cell Autoantigen.* Cell, 2019. **177**(6): p. 1583-1599.e16.

Long noncoding RNA MALAT1 promotes hepatic steatosis in HepG2 cells by increasing target genes expression in presence of Exendin-4

Olfa Khalifa[1], Sama Ayoub[2], Khaoula Errafii[1], Abdelilah Arredouani[1].

[1] Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation Doha, Qatar.
[2] Weill Cornell Medicine Qatar, Qatar Foundation, Education City, Doha, Qatar.

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) has been linked to liver cell proliferation. However, its role in hepatic steatosis and lipid accumulation is unknown.
The purpose of this study was to investigate MALAT1's effects on hepatic lipid accumulation and potential targets in presence of GLP-1R agonist Exendin-4 (Ex-4) which are currently approved to treat type 2 diabetes, have the potential to improve steatosis and even steatohepatitis.
We established steatotic HepG2 cells by treating them overnight with 400 M oleic acid (OA). The transcriptomic profiling was performed using total RNA extracted from untreated, steatotic, and Ex-4-treated steatotic cells. We validated a subset of differentially expressed LncRNAs with qRT-PCR and identified the most significantly enriched cellular functions associated with the relevant lncRNAs among them MALAT1.
First, treatment with 200nM Ex-4 for 3h decreased the OA-induced lipid buildup considerably ($p<0.05$). Concurrently, Ex-4 significantly lowered the expression levels of De novo lipogenesis genes such as SREBP-1, SCD-1and ACC, fatty uptake and transport genes also were affected by MALAT1 knockdown. Moreover, knockdown of MALAT1 expression dramatically suppressed oleic-induced lipid in HepG2 cells. Furthermore, knocking down MALAT1 significantly reduces the expression of CD36 in steatosis HepG2 cells in the presence of Ex-4. Finally, the silencing of MALAT1 prevented hepatic lipid accumulation in HepG2 cells. In conclusion, this study suggested inhibition of MALAT1 has potential for the treatment of NAFLD.
Our findings imply that MALAT1 may play important role in the processes driving steatosis improvement in response to GLP-1R agonists and call for more functional research.

Theme: Big data, new technologies, and translational genomics

Title: Implication of organic cation transporter genes (*OCT-1 and OCT-3*) polymorphism on response to metformin efficacy in North Indian type 2 diabetic cases.

Syed Tasleem Raza*#[a,d] Saliha Rizvi[a,b] Zeba Siddiqi[c] Farzana Mahdi[a,d]

[a]Department of Personalized and Molecular Medicine, [b]Department of Biotechnology, [c]Department of Medicine, [d]Department of Biochemistry, Era's Lucknow Medical College and Hospital, Lucknow, India

Background & Rationale: Type 2 diabetes mellitus (T2DM) is a global public health concern and increasingly prevalent illness. A great variability is seen in efficacy of oral antidiabetic drug metformin between patients owing to their unique genetic makeup. Consequently, understanding the genetic pathways involved in pharmacodynamics of metformin can affect personalized treatment of T2DM

Aims & Objectives: Study was aimed to find clinical implication of genetic polymorphism in Organic cation transporter ***(****OCT1 and OCT3)* genes on efficacy of metformin in T2DM cases.

Material & Methods: In this study, we evaluated the role of *OCT1 (rs628031)* and *OCT3 (rs rs2292334)* polymorphism on metformin response on T2DM cases. Response to metformin was defined by HbA1c levels based on which patients (n = 177) were divided into two groups: responders with HbA1C<7% (n = 127) and non-responders with HbA1C≥7% (n = 50). The responders were further subcategorized as T2DM cases on Metformin monotherapy (n = 55) and metformin with sulphonyl urea combination therapy (n = 72). Genotyping was done using PCR-RFLP approach. Odds ratios and 95% confidence intervals were calculated by logistic regression to assess the relative association between disease and genotypes.

Results: No significant association was found between *OCT1 (rs628031)* polymorphism and metformin response. On the other hand, significant association of *OCT3 (rs rs2292334)* polymorphism was observed with metformin response where AA genotype carriers showed higher efficacy of metformin both in mono [OR (CI) = 0.29(0.11-0.72) and p=0.007] and

combination therapy [OR (CI) = 0.41(0.16-1.0) and p=0.047]. In addition, A allele was more prevalent in responders [OR (CI) = 0.48(0.28-0.84) and p=0.010] while G allele was found to be associated with inefficacy of metformin in T2DM cases [OR (CI) = 2.07(1.19-3.61) and p=0.010].

Conclusion: Genotyping of *OCT3 (rs2292334)* might be useful in predicting the response to metformin in T2DM cases.

Title: Implication of organic cation transporter genes (*OCT-1 and OCT-3*) polymorphism on response to metformin efficacy in North Indian type 2 diabetic cases.

Syed Tasleem Raza*#[a,d] Saliha Rizvi[a,b] Zeba Siddiqi[c] Farzana Mahdi[a,d]

[a]Department of Personalized and Molecular Medicine, [b]Department of Biotechnology, [c]Department of Medicine, [d]Department of Biochemistry, Era's Lucknow Medical College and Hospital, Lucknow, India

Background & Rationale: Type 2 diabetes mellitus (T2DM) is a global public health concern and increasingly prevalent illness. A great variability is seen in efficacy of oral antidiabetic drug metformin between patients owing to their unique genetic makeup. Consequently, understanding the genetic pathways involved in pharmacodynamics of metformin can affect personalized treatment of T2DM

Aims & Objectives: Study was aimed to find clinical implication of genetic polymorphism in Organic cation transporter ***(****OCT1 and OCT3)* genes on efficacy of metformin in T2DM cases.

Material & Methods: In this study, we evaluated the role of *OCT1 (rs628031)* and *OCT3 (rs rs2292334)* polymorphism on metformin response on T2DM cases. Response to metformin was defined by HbA1c levels based on which patients (n = 177) were divided into two groups: responders with HbA1C<7% (n = 127) and non-responders with HbA1C≥7% (n = 50). The responders were further subcategorized as T2DM cases on Metformin monotherapy (n = 55) and metformin with sulphonyl urea combination therapy (n = 72). Genotyping was done using PCR-RFLP approach. Odds ratios and 95% confidence intervals were calculated by logistic regression to assess the relative association between disease and genotypes.

Results: No significant association was found between *OCT1 (rs628031)* polymorphism and metformin response. On the other hand, significant association of *OCT3 (rs rs2292334)* polymorphism was observed with metformin response where AA genotype carriers showed higher efficacy of metformin both in mono [OR (CI) = 0.29(0.11-0.72) and p=0.007] and

combination therapy [OR (CI) = 0.41(0.16-1.0) and p=0.047]. In addition, A allele was more prevalent in responders [OR (CI) = 0.48(0.28-0.84) and p=0.010] while G allele was found to be associated with inefficacy of metformin in T2DM cases [OR (CI) = 2.07(1.19-3.61) and p=0.010].

Conclusion: Genotyping of *OCT3 (rs2292334)* might be useful in predicting the response to metformin in T2DM cases.

SIDRA MEDICINE ZEBRAFISH FUNCTIONAL GENOMICS CORE FACILITY

Waseem Hasan[1]*, Doua Abdelrahman[1]*, Ahmad Hani[1], Stephan Lorenz[1], Rashid Alali[1], Khalid Fakhro[1,2, 3], Sahar Isa Da'as[1,2#]

[1]Sidra Medicine, Research Branch, Qatar. [2]College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar. [3]Weill Cornell Medicine, Qatar.

The Sidra Medicine Zebrafish Facility serves a significant role in developing clinically relevant zebrafish models of human genetic disorders and their related pathologies. The facility offers a platform with the state-of-the-art technologies and equipment to study the genetic variation impact on cell, tissue, organ, and whole organism levels. The facility provides access to zebrafish model, techniques, and tools to utilize the living fish model to its fullest potential.

Successfully, the facility validated patient-specific genotype models, which have provided a complete understanding of genetic variants, clinical manifestations, and disease prognosis. We currently offer pediatric disease models for Neurological disorders, Cardiovascular diseases, Congenital anomalies, diabetes, and liver diseases. Furthermore, we validated xenotransplantation of human stem cells, associated with pediatric conditions, from Sidra Medicine clinics into live zebrafish to study their proliferation and metastasis.

Our functional assays illustrate how zebrafish facility platform facilitate rapid translation into the clinic. The interpretation of clinical, genetic, genomic, and phenotypic data allowed researchers and clinicians to gain insights into the pathogenic outcomes of a human genetic variations and the interpretation of data provided by the Qatar Genome Project. To date, we have validated a variety of human genetic disorders that successfully contributed to 10 articles published in peer-reviewed journals.

Keyword**:** Zebrafish Platform, Human Disease Modeling, Functional Genomics

Role of *wars1* in apoptosis and its association with tumor metastasis.

Tayyiba Akbar Ali[a*], Mahmoud Izadi[a], Aya Nour[a], Ehsan Pourkarimi[a#]

[a] College of Health and Life Sciences, Hamad Bin Khalifa University, Education City, Doha 34110, Qatar

Faithful mRNA translation requires a family of enzymes called Aminoacyl tRNA synthetases (AaRSs) to ensure correct amino acids loading to their cognate tRNA, a step that occurs at the apex of the translation machinery. Among AaRSs, mutations in tryptophanyl tRNA synthetase (WARS1), required for loading tryptophan to its cognate tRNA, is highly linked with cancer. However, the exact mechanism of *wars1* mutations contributing to tumor progression and metastasis remains elusive. To this end, we set out to characterize the effects of *wars1* knockdown in *Caenorhabditis elegans* (*C. elegans)*. Using cytological markers and high-resolution microscopy we have shown that *wars1* knockdown causes caspase-depended apoptotic cell death in the germline of *C. elegans*. We also have confirmed that the increase in apoptosis upon knocking down *wars1* is not related to DNA damage. Apoptosis is an essential process required for the proper physiological cell function. The loss of apoptosis causes increased cell proliferation, leading to tumor formation. According to the cBioPortal database for cancer genomics, various gain of function mutations or gene duplication of *wars1* are associated with tumor progression and metastasis in various cancers such as lung, breast, and ovarian. Our study aims to functionally characterize the identified cancer-causing variants of *wars1* and their effect on apoptosis in *C. elegans* to understand cancer pathology and potential treatments. This will be achieved through Humanized *C. elegans* overexpressing the identified *wars1* variants generated using biolistic transformation. Thus, understanding the role of *wars1* in apoptosis will provide comprehension of targeted personalized chemo-treatments to patients carrying *wars1* mutations. All in all, this will ultimately decrease the healthcare burden.

PMFG 2022 theme: Big data, new technologies, and translational genomics

Novel protein-protein interactions in a set of human cancer pathway proteins

Stephanie Schaefer-Ramadan[a#*], Yue Guan[a], Jovana Aleksic[a] and Joel A. Malek[a]

[a]Department of Genetic Medicine, Weill Cornell Medicine in Qatar, Doha 24144, Qatar.
*Presenting author
#To whom correspondence should be addressed.
Tel: +974 4492 8465
Email: SAR2038@qatar-med.cornell.edu

Abstract
Objective: Identify novel protein-protein interactions in a set of human cancer pathway proteins.

Methods: We utilized a next-generation sequencing-based protein-protein interaction method developed in our laboratory called AVA-Seq (all-vs-all sequencing) based on a modified bacterial two-hybrid system. We tested all combinations of 1485 synthesized fragments from 91 human cancer-related proteins pair-wise. Protein-protein interactions among regions of the 91 proteins were identified in replicate experiments using mild and stringent selective conditions.

Results: Here, we report large numbers of significant novel protein-protein interactions between well-studied, cancer-related proteins. These interactions were recovered in replicate experiments, and many of these have overlapping unique protein fragment combinations suggesting the interaction contact point has been localized. We detected novel interactions and their interacting domain among wildtype p53, MCM2, and MCM3, whereas most other studies utilize a p53 mutation. Further novel interactions were observed, including the recently discovered COL18A:EGFR interaction detected in MDA-MB-231 and JIMT-1 cancer cell lines (Biorxiv 2022.01.10.474416). Novel cancer protein interactions could have implications for fundamental changes in multiple cancers. Of interest is the high number of novel interactions detected despite these proteins being well studied in cancer, suggesting the enormous potential for future findings. As we delve further into the dataset, we will elucidate protein contact regions in known and novel protein interactions allowing more targeted drug design. Further work will also include comparing protein interactomes of wildtype and variant cancer pathway proteins by introducing cancer-specific polymorphisms to the tested fragments which could aid in precision medicine for specific cancer variants. Our results show the potential for significantly increasing cancer protein interaction information by utilizing the highly sensitive AVA-seq system.

Conclusion: We provide a high-resolution interactome of cancer-relevant proteins that could direct novel drug targets and implicate possible effects of specific cancer mutations.

Blood sphingolipid profiling shows altered metabolism in obese and lean asthmatic children from the MENA region

Harshita Shailesh[a]*, Safa Noor[a], Mohammad Alfaki[a], Yahya Hani[a], Lena Hasabelgawi[a], Salma Ahmed[a], Saroja Balayya[b,] Shana Jacob[b], Tilla S Worgall[c], Stefan Worgall[d], Ibrahim Janahi[e#]

a Department of Medical Services, Sidra Medicine, Doha Qatar, b Advanced Cell Therapy Core, Sidra Medicine, Doha Qatar, c Department of Pathology and Cell Biology, Columbia University Medical Center, New York, d Department of Pediatrics, Weill Cornell Medicine, New York, e Department of Pediatric Pulmonology, Sidra Medicine

Asthma is one of the most common significant chronic diseases affecting children worldwide and children with obesity are at an increased risk for developing asthma. Studies have shown that obesity alters both early onset atopic asthma and leads to the development of late-onset non-atopic asthma. Dysregulated sphingolipid metabolism has been associated with airway hyperreactivity and asthma. The aim of this study was to investigate whether sphingolipid metabolism is altered in childhood obesity-related atopic and non-atopic asthma.

Blood samples from five study groups, lean-atopic asthmatic, obese-atopic asthmatic, obese non-atopic asthmatic, obese non-asthmatic, and healthy controls were collected (n=10 each, age 6-18 years, Qatar residents). Erythrocytes from these blood samples were analyzed to determine the concentration of 26 individual sphingolipids, including sphingomyelins (SM), ceramides (Cer), and dihydroceramides (DhCer) by liquid-chromatography-triple quadrupole mass spectrometry.

The sphingolipid concentration values were analyzed by single-factor ANOVA and those lipids with $p<0.05$ were analyzed further by t-test. Significant differences in certain sphingolipids were found between atopic-obese asthma compared to healthy control (24:1SM, 24Cer, 24DhCer, and 24:1Cer $p<0.05$; 16:1SM and 24:1DhCer $p<0.005$) while no significant differences were found between non-atopic obese asthma and healthy control. Additionally, several significant differences were found between obese-atopic asthma and lean-atopic asthma group, of which, 24Cer and 24:1DhCer were of most importance ($p<0.0005$). These same two lipids were not significantly different when comparing obese non-asthmatic and healthy control.

The results of our study indicate that sphingolipid metabolism is altered in different types of pediatric asthma. Atopic-obese asthma and non-atopic obese asthma show different sphingolipid profiles and the levels of 24Cer and 24:1DhCer are affected by having obesity and atopic asthma in combination. These altered sphingolipid profiles indicate that these lipids may serve as potential biomarkers and targets for developing personalized medicine for different asthma endotypes.

Abstract for PMFG 2022

Title

Leveraging orthogonal sequencing and optical mapping technologies for the precision diagnosis of neuro-developmental disorders in a Middle Eastern family-based cohort

Authors

Zenab Siddig[1], Dania Hammadi[1], Aya Noor[1], Sondoss Hassan[1], Mohammadmersad Ghorbani[1], Haroon Naeem[1], Ilhame Diboun[1], Shabir Moosa[1], Rawan Abouelhassan[1], Melissa Tauro[1], Sara Nassar[1], Radi Farhad[1], Rozaimi Razali[2], Iman Alazwani[4], Muna Al Hashmi[4], Rebecca Matthew[4], Li Liu[4], Guishuang Wang[4], Shafeeq Poolat[3], Ahmad El Khouly[3], Sara Tomei[4], Stephan Lorenz[4], Donald Love[5], Basma Selim[6], Khalid Fakhro[7,9,10], Alia Satti[6,*], Ruba Benini[8,*], Younes Mokrab[1,9,10]*

Affiliations

1 Medical and Population Genetics Lab, Human Genetics Department, Research Division, Sidra Medicine, Doha, Qatar
2 Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar
3 Research IT Infrastructure, Sidra Medicine, Doha, Qatar
4 Integrated Genomics Services, Sidra Medicine, Doha, Qatar
5 Pathology Division, Sidra Medicine, Doha, Qatar
6 Developmental Pediatrics Division, Sidra Medicine, Doha, Qatar
7 Genomic Medicine Lab, Human Genetics Department, Research Division, Sidra Medicine, Doha, Qatar
8 Neurology Division, Sidra Medicine, Doha Qatar
9 Department of Genetic Medicine, Weill Cornell Medicine, New York, U.S.A.
10 College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar
* Corresponding authors. Email: ymokrab@sidra.org

Abstract

Establishing genotype-phenotype correlations for neurodevelopmental disorders (NDDs) is a substantial challenge, especially amongst Middle Eastern populations enriched for these conditions due to high level of consanguinity. We implemented a platform integrating data from short and long read WGS, optical mapping and EHRs to diagnose and characterize unresolved pediatric cases with neurodevelopmental disorders in Qatar. In a pilot project, we recruited a cohort with intractable epilepsy or developmental delay with no genetic diagnosis, consisting of 114 families of patients. We generated Illumina WGS and PacBio long-read sequencing, from which we obtained high quality SNVs and Indels as well as SV and CNVs respectively. EHRs were collected and mapped to HPO from which the terms global developmental delay, delayed speech, and seizures were found to be most common. Family segregation genomic analysis was done using Congenica© using gene panels from the G2P consortium. Variants were annotated with allele frequency from Qatar Genome Project and other public datasets, as well as pathogenicity predictors including Exomiser and CADD. Candidate disease variants were then prioritized following ACMG guidelines. Using this approach, we analyzed a set of 12 families

leading to the shortlisting of 26 variants on 25 genes. Thirteen genes were known to be implicated in NDDs. Amongst the variants, 11 variants were previously reported including 9 with phenotypes reported as matching the patient's phenotypes. We identified 10 novel variants on known pathogenic genes. For instance, we identified a novel missense variant of uncertain significance (chr12:56565123) in *SMARCC2* in a 4-year-old female who exhibits global developmental delay, autism, and hypotonia. *SMARCC2* is associated with Coffin-Siris Syndrome-8 (CSS8) which is characterized by a range of developmental delays, hypotonia, and behavioural abnormalities. In 15 patients with CSS8, Machol et al. (2019) detected 13 heterozygous mutations in the *SMARCC2* gene, 7 of which were missense pathogenic variants. The evidence on pathogenicity of missense variants in *SMARCC2* and their associations with the patient's phenotypes indicates that this a strong candidate gene, and this variant is possibly a novel disease-causing variant. As we continue to analyze this patient cohort, the results will have significance impact on to the precision diagnosis and understanding of developmental disorders in the Middle East and worldwide.

Seven variants including two novel splice and one novel missense mutation in PHD domain justify the loss-of-function mechanism of *PHF21A* and extend the spectrum of *PHF21A* mutations

Afif Ben-Mahmoud[a*], Vijay Gupta[a], Lawrence W. Stanton[a], and Hyung-Goo Kim[a#]
a-Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar. * Presenting author # Corresponding author.

Three genes within the 2.1 Mb genomic critical region at 11p11.2 are the major determinants of Potocki-Shaffer-Syndrome (PSS), characterized by multiple exostoses, biparietal foramina, intellectual disability (ID), and cranio-facial anomalies (CFA). Among these three genes, we reported previously that *PHF21A* was associated with intellectual disability and craniofacial anomalies by the positional cloning of two patients with balanced translocations. *De novo* truncating mutations in three patients with ID and CFA were subsequently reported for the first time in Japanese patients. More recently, the phenotypic spectrum of *PHF21A* mutations has been extended by us in seven patients with ID and CFA by adding autism spectrum disorder, epilepsy, hypotonia, and neurobehavioral problems. Furthermore, we provide eight additional patients with *de novo* mutations in *PHF21A* and detailed clinical features including ID/DD and CFA. Among them, two patients provide the first aberrant splicing mutations in *PHF21A*. These variants causing premature frameshift mutations in RT-PCR resulted in truncated proteins, which supports the previous hypothesis of loss-of-function as underlying mechanism. Furthermore, we have identified, for the first time, a missense mutation in a PHD domain, essential for binding H3K4me0. Since the underlying mechanism of *PHF21A* mutations is haploinsufficiency, this patient emphasizes that a point mutation in PHD domain alone

is sufficient to inactivate the whole function of *PHF21A* as was seen in the point mutation in AT-Hook domain we reported before. This emphasizes the vital role of PHD and AT-Hook domains in the molecular function of *PHF21A*.

Distribution of variants affecting response to TNF inhibitors in the Qatari population

Zainab Jan*, Borbala Mifsud, Puthen Veettil Jithesh#

College of Health & Life Science, Hamad Bin Khalifa University, Education City, Qatar

* Presenting author; #Corresponding author (jveettil@hbku.edu.qa)

Autoimmune diseases are increasing globally, causing mortality and morbidity worldwide. Over the past few decades, TNF inhibitors that target tumor necrosis factor (TNF-α) have been widely used for the treatment of autoimmune diseases. However, 40% of the patients do not respond to TNF inhibitors. Several pharmacogenetic variants affecting TNF inhibitors response have been identified. However, the prevalence of these variants in the Qatari population is still not known.

In this study, we identified the distribution of known and novel variants in 102 genes associated with response to TNF inhibitors in 14,392 Qataris from whole genome sequencing data.

Out of the 119 known pharmacogenomic variants associated with response to TNF inhibitors, ~90% had different allele frequency distribution from other world populations present in the gnomAD dataset. High frequency of rs1143634 (*IL1B)* and rs1800896 (*IL10)* variants was observed, that are known to be associated with both negative and positive responses to infliximab and etanercept respectively. Moreover, we identified that the allele frequency of rs1295686 (*TH2LCRR*) associated with better response to Adalimumab is higher in the Qatari population. Furthermore, higher distribution of AA genotype on *HLA-E* (rs1264457) gene was observed that showed better response to all TNF inhibitors.

The findings of this study could be the basis for developing and implementing pharmacogenetic testing in autoimmune disease patients being considered for TNF inhibitor therapy in Qatar and beyond.

KDM5D may contribute as a critical regulator to orchestrate Y-chromosome encoding genes in acute lymphoblastic leukemia

Azin Keshavarz[a]*, Sara Chitgaran[b], Fatemeh Nasrabadi[a], Saeed Khatami[b], Vahid Moghimi[a], Reza Alemohammad[a] Ali Ghasemi [c], Moein Farshchian[a]#

[a] Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture, and Research (ACECR), Khorasan Razavi, Mashhad, Iran.

[b] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[c] Department of Pediatric Oncology-Hematology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

moein.farshchian@mail.um.ac.ir

Acute Lymphoblastic Leukemia (ALL) is one of the most widespread malignancies in the pediatric population. The KDM protein family which encodes several histone lysine demethylases plays a part in ALL, but their interactions with other genes and contributions to tumorigenesis remain unknown. In recent years, Next Generation Sequencing (NGS) technologies have provided comprehensive insight into transcriptomics. In this study, we assessed genome-wide co-expression profiling in ALL patients to reveal the hidden interplay between distinct gene clusters. The project has been carried out using public datasets available in NCBI. The accumulated number of samples is 292, consisting of 250 B-ALL patients and 42 normal samples. Transcriptome profiling was performed using the DESeq2 package. Further downstream analysis was conducted in the WGCNA (Weighted Gene Co-expression Network Analysis) package in R. Network analysis showed that *KDM5D* had the highest weight (~0.7) among all the modules constructed by WGCNA and also, and the majority of the genes associated with *KDM5D*, were located in the Y chromosome. Moreover, *KDM5D* was also highly expressed in ALL samples. Taken together, despite we could not showed a mechanistic approach for regulation of sex-related genes by this epigenetic factor but our results revealed that *KDM5D* may have a tumorigenesis role in contributing to the regulation of Y-chromosome encoding genes.

Obese Asthmatic children show different metabolomics profiles than lean asthmatic children

Mohamed Alfaki[a*], Harshita Shailesh[a], Safa Noor[a], Yahya Hani[a], Lena Hasabelgawi[a], Salma Ahmed[a], Ilhame Diboun[b], Shana Jacob[c], Till S Worgall [d], Stephan Worgall[d], Ibrahim Janahi[a,e#]

[a]Department of Medical Services, Sidra Medicine, Doha, Qatar, [b]Translational Genetics Department, Sidra Medicine, Doha, Qatar, [c]Advanced Cell Therapy Core, Sidra Medicine, Doha, Qatar, [d]Department of Pediatrics, Weill Cornell Medicine, New York, USA, [e]Department of Pediatric Pulmonology, Sidra Medicine, Doha, Qatar

Asthma is one of the most chronic respiratory diseases among children worldwide and obesity is recognized as one of the important risk factors for developing asthma in children. Obesity is extensively associated with metabolic dysregulation and is considered to play a crucial role in the pathogenesis of different diseases including asthma. Metabolomics profiling has emerged as a new tool to identify biological markers for precision and personalized medicine. This study was aimed to explore different metabolites associated with obesity-associated asthma in the pediatric population of the MENA region. In this study, untargeted metabolomic profiling was performed using plasma of blood samples from asthmatic lean (n = 10), asthmatic obese (n = 10), non-asthmatic obese (n = 10), and non-asthmatic lean (n = 10) children by liquid chromatography-high resolution mass spectrometry. The data obtained was analyzed by principle component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Identification of differential metabolites between different study groups was made by in-house libraries and online databases with 5ppm mass tolerance. Biomarker identification and pathway enrichment analysis were performed on the top discriminant metabolites with p-value<0.05.

Significant differences in metabolites were found between obese asthmatic children compared to both asthmatic lean and non-asthmatic obese groups with $p<0.05$. Three metabolites including urate-3-ribonucleoside, L-alpha-Aminocaprylic acid, and guanosine were found to be different when comparing the asthmatic lean with the asthmatic obese groups. Pathway enrichment analysis revealed that these metabolites were enriched in phenylalanine and tyrosine metabolism. Furthermore, 4 metabolites including N-Acetyl-L-citrulline, Pyrazole-1-alanine, Capryloylglycine, and Hypoglycin A found to be significantly different between asthmatic obese from the non-asthmatic obese children.

Our findings suggest that obese asthmatic children are characterized by a metabolic profile different from the lean asthmatic. These metabolites could serve as possible personalized biomarkers and therapeutic targets in the pediatric population of the MENA region.

Mucin 16, Cell Surface Associated Expression and its prognostic significance in ovarian cancer

Suhila Alkhair[1#], Ola Taha[2]

[1]Faculty of Medicine, alzaiem alazhari university, Khartoum, Sudan.

[2]Faculty of Medicine, University of Khartoum, Khartoum, Sudan.

Introduction

Ovarian cancer (OV) is one of the top death causes in the gynecological malignancies, it affects women at all ages but due to the symptoms at late stage most of the women diagnosed in the last stage with less curation rate. Studies showed that Muc16 is a large transmembrane protein that expressed in almost 80% of epithelial ovarian cancer, the immune protection provided by MUC16 (a carrier of the tumor marker CA125) may lead to selective survival of ovarian cancer cells that are more efficient in metastasis. The aim of this study was to understand the role of MUC16 comprehensively using computational approach.

Method

MUC16 expression and its influence on tumor prognosis were analyzed by the Tumor Immune Estimation Resource (TIMER) and Kaplan-Meier plotter. The relationship between MUC16 expression and tumor immunity were analyzed by TIMER and Gene Expression Profiling Interactive Analysis (GEPIA), furthermore, the mutation of MUC16 in OV was analyzed by cBioportal.

Results

The overall expression of MUC16 was higher in ovarian cancer compared to other type of cancers, MUC16 expression was high in OV patients compared to the normal patients, the expression was elevated in patient at age range between 21 – 40 years and ovarian cancer tumor grade three and stage four.

Conclusion

Our work concludes that MUC16 is upregulated in OV samples and these findings demonstrate that MUC16 is a potential prognostic biomarker that determines cancer progression and correlated with tumor immune cells infiltration in ovarian cancer. Determining the detailed biological function of MUC16 in OV tissues will provide promising and insightful information for further study.

Keywords: MUC16, cancer, ovarian cancer, Biomarkers.

DIET INTERACTION WITH GUT MICROBIOME AND SERUM LIPIDOME PROFILING IN A PILOT STUDY ON THE OBESE QATARI FEMALE POPULATION

Salma Hayder Ahmed[1] , Arun Prasath Lakshmanan[1,*], Shana Jacob[2], Shaikha Al Abduljabbar[1], Sahar Al-Shamari[3], Wahiba Elhag[3], Yassmin Salaheldin Mohamed Ibrahim[3], Annalisa Terranegra[1,#]

[1]Precision Nutrition, Research Department, Sidra Medicine, Doha, Qatar.
[2]Advanced Cell Therapy Core, Research Department, Sidra Medicine, Doha, Qatar.
[3]Bariatric and Metabolic Surgery Department, Hamad Medical Corporation, Doha, Qatar.

[*]Presenting author: Annalisa Terranegra, aterranegra@sidra.org

Abstract

Obesity is a complex disease with underlying genetic, environmental, and epigenetic factors, etc. Obesity is a risk factor for various disorders in women, including cardiovascular diseases, diabetes, infertility, pregnancy complications, that are among the most prevalent chronic conditions in Qatar. Also, according to data released by MOPH in Qatar, the obesity prevalence rate is higher in women than men in Qatar. Recent studies have highlighted the significant roles of the gut microbiome and serum lipid metabolites in contributing to the pathology of obesity. Thus, in this study, we aimed to identify the interaction of diet with microbial and lipidomic biomarkers in an obese Qatari female population.

We enrolled 35 adult female subjects (18-65 years), and they were classified based on their BMI, in Obesity-I group (<35 kg/m^2, n=18), and Obesity-II and -III group (>35 kg/m^2, n=17). For each subject, we measured anthropometric data, body composition, biochemical profile, dietary intake using 24hrs dietary recalls, gut microbial composition by 16s rDNA sequencing method that targeted the v3-v4 region using Illumina Miseq and serum lipid metabolites using liquid chromatography - high resolution mass spectrometry (LC-HRMS). Bacterial diversity index, microbial markers, and correlation analysis were performed.

The results were analyzed by comparing 2 groups: Obesity-I vs Obesity-II and III together. The dietary data showed a positive correlation of vitamin D and total sugars intake with basal metabolic rate (R=0.488, p=0.007, R=0.382, p=0.037, respectively) and multiple body composition parameters. The taxonomic and lipidomic profiling showed that Obesity-II & -III group had a higher abundance of genera *Streptococcus* (8.90% vs 24.09%, p=0.04.) and unclassified bacteria from the class of TM7-3 (0.35% vs 0.86, p=0.02), a higher level of a mono-hexosylceramide metabolite - Hex1Cer(t18:0_24:1) (3.04x10^7 vs 2.49x10^7, area under the pick, p=0.005) and lower levels of different phosphatidylcholines, lysophosphatidylcholines and phosphatidylethanolamine metabolites. Also, correlation analysis revealed that the genera *Streptococcus* and unclassified bacteria from the class of TM7-3 were positively correlated with body weight (BWT, R=0.487, p=0.04 (*Streptococcus*), R=0.367, p=0.004 (Unclassified bacteria from the class of TM7-3), muscle mass (MM, R=0.416, p=0.016 (*Streptococcus*), dietary MuFA (R=0.641, p<0.0001), PuFA (R=0.415, p=0.0035), galactose (R=0.428, p=0.029), sucrose (R=0.448, p=0.022), and available carbohydrates R=0.548, p=0.004).

Controlling the dietary intake, particularly the intake of fats and sugars, can contribute to modulate the genus *Streptococcus* and the ceramide metabolite Hex1Cer(t18:0_24:1) playing an unprecedented role in the management of severe obesity in the Obese Qatari female population.

I like to present my work as oral presentation / poster in the following session:

Session 2 – Precision Nutrition (Chairs: Lorraine Brennan, Lydia Afman)

Treatment of acrodermatitis continua of Hallopeau during pregnancy using targeted therapy. Sara Al-Khawaga, MD,[*,#, a,b] Roopesh Krishnankutty, PhD,[c] Gulab Sher, PhD, [c] Khairunnisa Hussain, MD,[a] Joerg Buddenkotte, MD, PhD,[a,b] and Martin Steinhoff, MD, PhD,[*,#, a,b]

[a] Department of Dermatology, Hamad Medical Corporation, Doha, Qatar
[b] Dermatology Institute, Hamad Medical Corporation, Doha, Qatar
[c] Translational Research Institute, Hamad Medical Corporation, Doha, Qatar
[d] Weill Cornell Medicine-Qatar, Doha, Qatar.
[e] Weill Cornell Medicine-New York, New York, USA
[f] Qatar University, Medical School, Doha, Qatar.
* Presenting authors
# Corresponding authors

Background. Acrodermatitis continua of Hallopeau (ACH), a form of localized pustular psoriasis is often recalcitrant to therapy. A variety of local and systemic therapies have been utilized for ACH with variable results. No standardized guidelines have been developed for ACH management as of yet. In the present case, a 23-year-old woman was diagnosed with ACH. The 2-year treatment course of the patient presenting with persistent, scaly, erythematous, pustular, and tender plaques on the distal portion of the digits, was successful.

Results. Our proteomics-based analysis resulted in comprehensive profiling of plasma proteins before and after therapy with guselkumab and certolizumab, respectively. Comparative plasma proteome profiling revealed marked down-regulation of distinct biological pathways such as complement activation, innate immune response, inflammatory response and lipoprotein metabolism. In particular, apolipoproteins were found reduced in plasma under therapy. Cytokine/chemokine profiling supported a therapy-dependent decline in plasma levels of innate immune mediators such as IL-6, TNF-α and CXCL1 confirming our MS-based observation of an attenuated innate immune response after therapy.

Conclusion. To our knowledge, our case demonstrates for the first time an excellent long-term response to improve ACH using either guselkumab or certolizumab. This is further the first proteomic profiling in a patient with ACH under therapy. More precision medicine-based studies and controlled clinical trials are necessary to understand pustular psoriasis and its sub-form ACH, and may lead to novel safe, and efficacious therapies.

Prediction of key signaling pathways based on H&E images in head and neck squamous cell carcinoma using deep learning

Tariq Masoodi[1*#], Assif Assad[2], Muzafar Rasool Bhat[2], Tabasum Majeed[2], Syed Wajid Aalam[2], Shahab Uddin[3,4,5], Ammira Al-Shabeeb Akil[6], Muzafar A. Macha[2], Ajaz A. Bhat[6]
[1]Human Immunology Department, Research Branch, Sidra Medicine, Doha, Qatar
[2]Islamic University of Science and Technology, Jammu & Kashmir, India
[3]Translational Research Institute,Academic Health System, Hamad Medical Corporation, Doha, Qatar
[4]Laboratory of Animal Research Center, Qatar University, Doha, Qatar
[5]Dermatology Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar
[6]Department of Human Genetics-Precision Medicine In Diabetes, Obesity and Cancer Program, Sidra Medicine, Doha, Qatar

Abstract

Deregulation of key signaling pathways in head and neck squamous cell carcinoma (HNSCC) contributes to drug resistance and poor patient prognosis. Identification of these signaling pathways show promise in specialized and personalized targeted therapies for the patients but require laborious genomic analysis like high-throughput molecular profiling. We used artificial intelligence (AI) based deep learning to read histopathological patterns in hematoxylin and eosin-stained histopathology slide images of HNSCC and correlate these with six known biological pathways. We downloaded 510 whole-slide images (WSIs) from the Genomic Data Commons database along with genomic and clinical data to identify the altered pathways and train the AI model. The WSIs were divided into numerous homogenous 512x512 size titles (median 10397, range 5000-30000) with each title sharing the same label as the parent image to train the Deep Convolutional Neural Network (DCNN). Each tile is fed as input to the pre-trained Deep Learning (DL) model (InceptionV3) to extract a 2048-dimensional feature vector. Feature vectors of all tiles of the same patient are stacked and fed into a DL Feed Forward Neural Network (FFNN) model to predict the outcome. The model precisely predicted the canonical signaling pathways of HNSCC with robust accuracy for Epithelial-Mesenchymal Transition Pathway (accuracy 68%, AUC score 0.68), PTEN Signaling (accuracy 66%, AUC score 0.64), JAK/STAT Signaling (accuracy 67%, AUC score 0.67), PI3K/AKT Signaling (accuracy 63%, AUC score 0.63), p53 Signaling (accuracy 69%, AUC score 0.68) and ERK/MAPK Signaling (accuracy 61%, AUC score 0.60).

The results provide insights into the association between WSI features and biological signaling pathways in HNSCC. These findings show the remarkable potential of AI based Deep Learning model in characterizing the signaling pathways directly from H&E images which is clinically important, where personalized therapeutic interventions may potentially improve patient outcomes.

Investigating and identifying monogenic variants as underlying cause for hyper IgE syndrome in four families

Randa AlYafie[§a,b,*], Taushif Khan[b], Manar Ata[b], Fatima Al-Ali[b], Evonne Chin-Smith[b], Salim Bougarn[b], Mohammed Toufiq[b], Nico Marr[ψa,b], Puthen Veettil Jithesh[ψa], Nicholas Panhuys[ψb], Mehdi Adeli[¶b,c] and Andrea Guennoun[¶b#]

a College of Health and Life Sciences, Hamad bin Khalifa University, Doha, Qatar
b Research Department, Sidra Medicine, Doha, Qatar
c Department of Allergy and Immunology, Sidra Medicine, and Hamad Medical Corporation, Doha, Qatar

* Presenting author
# Corresponding author
§, ψ , ¶ equal contribution

Hyper-IgE syndromes (HIES) are a subset of primary immunodeficiency diseases characterized by persistent skin abscesses, dermatitis, allergies, and pulmonary infections, as well as high serum IgE levels. Both, autosomal dominant (AD) and recessive (AR) forms of the disease were reported. While the prevalence of HIES is rare, in countries with high rates of consanguinity and large family sizes, a higher prevalence, in particular of AR cases, is observed.

We enrolled four pediatric patients who suffer from severe eczema, multiple allergies, severe and/or recurrent infections and markedly elevated IgE levels since infancy. We hypothesized that the patients carry genetic mutations that lead to HIES.

The National Institutes of Health scoring system was applied to two of the patients and returned scores above the threshold, suggesting HIES as the underlying condition. Further, all patients and family members, underwent whole genome sequencing. In one patient born to consanguineous parents, we predicted a novel homozygous missense mutation (c.1066G>A, p.Ala356Thr) in PGM3 which is private to the family. A second, consanguineous patient carries a novel, private, AD missense mutation (c.2913C>G, p.Cys971Trp) in CARD11, with mild loss-of-function and dominant-negative activity in a NF-kB-reporter assay. Further, a large-scale antibody screen, by phage immunoprecipitation-sequencing, was conducted to assess the patients' infection history and revealed overall normal IgG antibody responses to common respiratory viruses in two tested patients.

While mutations in PGM3 and CARD11 were reported before as underlying monogenic causes of HIES and may explain the clinical phenotype of two patients, no candidate gene was identified in the remaining two cases. Consequently, large structural variant analysis with AnnotSV is ongoing as large deletions e.g. in DOCK8, were reported as causative for HIES. Additional in silico and functional investigations are presently being conducted to evaluate causal links between candidate genes and the immunological and clinical phenotypes.

Risk and Occurrence of Diabetic Retinopathy in Children and Adolescents with Type 1 Diabetes: Retrospective Results from Health Medical Records

Reem Al-Karbi [1], Mubarak Ziab [1], Jyothi Laksmi, Ikhlak Ahmed [1], Abdul Rauf Kamboh [2], Abdallah Mahmoud [2], Ammira Alshabeeb Akil [1*]

[1] Precision Medicine of Diabetes, Obesity and Cancer Research group, Precision Medicine Program, Department of Human Genetics, Sidra Medicine, P.O. Box 26999, Doha, Qatar

[2] Department of Pediatric Ophthalmology, Sidra Medicine, P.O. Box 26999, Doha, Qatar

[*] Corresponding author: Ammira Akil, email: aakil@sidra.org

Abstract:

Diabetic retinopathy (DR) is one of the main diabetes complications that affects the retina and might lead to blindness in advanced stages. The primary aim of this study is to investigate the prevalence rate of this complications in the affected children in addition to emphasize the importance of early and annual screening of DR in diabetic children and adolescents of Qatar.

Study design: a retrospective study designed to determine the prevalence of DR in diabetic children and adolescents living in Qatar. Data presented in the current study has been collected retrospectively from accessing medical health records (Cerner) of (355) diabetic patients with Type 1 diabetes or Type 2 diabetes, aged 4 to 21, who visited Sidra Medicine ophthalmology clinics between year 2017 and 2022 (IRB 1707012119). The retinas of diabetic children were examined by the ophthalmologist using fundus photography and graded based on the retinopathy grading scale.

Preliminary data: Out of 355 patients, 327 were with T1D patients, and 28 were T2D. Based on the current screened number, the prevalence of DR in children estimated to be 3.55% (10 cases of DR out of 355 screened participants). Notably, all those currently diagnosed with DR were T1D patients and are in the older age group of the screened cohort (15-19 years of age), with an average duration of T1D as 13.6 years. The ophthalmologist graded all DR patients as R1, which indicates background retinopathy, (the earliest stage of DR).

Conclusion: at the current stage of screening, we found relatively lower prevalence of DR in children and adolescents with diabetes than previously reported in other populations. However, this expected to be changed with accessing more patients and collecting further data. The incidence of DR was higher among patients with T1D and occurred with a longer duration of disease. While the incidence of DR in youth with T1D is higher, with the increasing incidence of T2D in adolescents and early risk for DR, early screening must be emphasized.

Integrative genomic and metabolomic approach to diagnose rare Mendelian diseases in Qatar

Rulan Shaath[1], Aljazi Al-Maraghi[1], Noha A. Yousri[2], Sasirekha Palaniswamy[1], Waleed Amer[1], Ammira Akil[1], Khalid Fakhro[1]

[1]Department of Human Genetics, Sidra Medicine, 26999, Qatar, [2] Genetic Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar

The growing large-scale adoption of genomic approaches for diagnosing rare Mendelian diseases is hampered by the low diagnostic yield where a great proportion of patients remain undiagnosed. Recently, integrative OMICs-approaches have been proven useful in closing this diagnostic gap. Metabolomics is an emerging field involving the characterization of small molecules known as metabolites and their association with particular traits or disease-related pathways. In this study, along with an extensive genomic analysis pipeline, we integrated metabolomic data from 20 patients with rare diseases, of which 35% did not have any known genetic cause, while 75% had a potential genetic cause. The establishment of the candidate metabolites was based on identifying those with extreme levels (Z-score threshold of ±2 standard deviation). Of a total of approximately 1000 metabolites per patient, we find an average of 63 with extreme value. These outliers were then prioritized by finding metabolic pathway(s) perturbations with relevance to the patient phenotype. Using this approach, we identified perturbation of ceramides, a precursor of sphingolipids that regulate cilia formation, as a potential biomarker in two patients with ciliary dysfunction. Moreover, integrating metabolomic analysis could complement and validate genetic findings. For instance, four sulfated steroids were downregulated in a patient with osteoarthritis, suggesting KCNG4, a voltage-gated potassium channel, as a novel candidate gene for osteoarthritis. Overall, the use of metabolomics alongside genomics shows promising results in diagnosing rare Mendelian diseases by identifying novel biomarkers, uncovering the pathophysiology of a disease, and most importantly, validating genetic results.

QATAR Type 1 Diabetes Biorepository: A Resource for Studies of the Genetic Risk, Autoimmunity Determinants and Etiologies of Type 1 Diabetes in Qatar

Saadya Amrullah[1], Ikhlak Ahmed[1], Goran Petrovski, Mohammed Bashir, Mubarak Ziab[1], Jyothi Lakshmi, Mariam Al-Maraghi, Amal Hussain, Sasirekha Palaniswamy[2], Sura Ahmed Hussain, Khalid Fakhro[1,2], Ammira Alshabeeb Akil[1,2‡]

[1]Department of Human Genetics, Precision Medicine Program in Diabetes, obesity, and Cancer, Sidra Medicine, 26999, Doha, Qatar; [2]Laboratory of genomic medicine, Precision Medicine Program, Sidra Medicine, 26999, Doha, Qatar

[‡]**Corresponding author: Ammira Alshabeeb Akil**
Precision Medicine in Diabetes, Obesity and Cancer Group
Precision Medicine Program, Human Genetics Department, Research Branch at Sidra Medicine

Abstract

Brief Background: Qatar has the 4th highest incidence of type 1 diabetes (T1D) worldwide. Given the dangers of T1D burden and the need for clinical and biomedical research nurturing around disease risks and etiologies, we took the initiative to establish "Qatar T1D biorepository" based on the experience and knowledge of global organizations and tailored to Qatar's needs.
Program Objectives: The objective of the protocol is to present the baseline data, design, and methods used for establishing Qatar T1D biorepository. Further aims include an assessment of the prevalence of T1D and performing deep population-specific investigations into the disease pathogenesis.
Design: Qatar T1D biorepository was primarily established in 2020 as a disease-specific biorepository containing peripheral blood, serum, plasma, buffy coat, and peripheral blood mononuclear cells (PBMCs) from patients with T1D and their first-degree relatives to serve as a control population. The biorepository is linked to the Sidra Medicine Precision Medicine Program and participants are enrolled by two main hospitals and healthcare providers in Doha, Qatar, including Sidra Medicine and Hamad Medical Corporation. New specimen storing and preservation, data management, and data sharing technologies have been used to advance a long-standing T1D-specific population biorepository with accounting and addressing all ethical considerations.
Collected data summary: Currently, the biorepository contains samples from around 130 T1D patients and 180 healthy controls from approximately 110 families. The mean age of patients enrolled is 11.7 ± 4.38 years, with the ratio of affected males to females of 0.9:1. The mean fasting blood glucose (FPG; mmol/l), HbA1c and BMI values are 17.11 ± 16.87 (min = 4.1; max = 86), 8.7 ± 2.08 (min = 5; max = 15) and 21.36 ± 5.41 (min = 13.38; max = 34.98), respectively. According to the BMI data, 14% of patients are overweight or obese. Of the 110 families, a total of 240 participants of 71 families have undergone whole-genome sequencing to identify known and novel pathogenic genetic variants associated with T1D.
Conclusion: building a disease-specific biorepository of clinically well-characterized biospecimens and data will advance T1D research for better translational outcomes. Qatar T1D biorepository built to be the largest T1D parent-case trio cohort in Qatar and in the region. The biorepository invites collaborative investigations of T1D genetic risks and novel immunological discoveries as well as predictive and prognostic biomarkers of autoimmunity.

Long non-coding RNA *RP11-109D20.2* as a novel biomarker in colorectal cancer

Sara Chitgaran[a*], Fatemeh Nasrabadi[a], Ehsan Soltani[b], Amir Nader Emami Razavi[c], Maryam M. Matin[a,d,e#], Moein Farshchian[e##]

[a] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
[b] Department of Cancer Surgery, Surgical Oncology Research Center, Mashhad University of Medical Sciences, Taleghani University Hospital, Mashhad, Iran
[c] Iran National Tumor Bank, Cancer Biology Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran
[d] Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
[e] Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi, Mashhad, Iran

matin@um.ac.ir
moein.farshchian@mail.um.ac.ir

Colorectal Cancer (CRC) is among the most notorious malignancies worldwide, which is particularly difficult to be detected in the early stages. It would be highly desirable to diagnose CRC patients at an early stage when there is still a chance for treatment. Therefore, it is important to search for novel biomarkers for early detection. In this study, we aimed to focus on a novel lncRNA and its possible role in CRC by interrogating the transcriptome through RNA Sequencing data. This project takes advantage of a publicly available dataset from NCBI (BioProject: PRJEB27536) consisting of 62 samples (tumors and adjacent normal tissues).

DESeq2 and WGCNA (Weighted Gene Co-expression Network Analysis) packages were used with the aim of profiling the differentially expressed lncRNAs and constructing co-expressed gene networks, respectively. Further functional analyses were performed using Gene Set Enrichment Analysis (GSEA) in order to find enriched pathways in CRC.

The network analysis revealed that among all the 23 modules constructed in this study, the most significant module (in terms of correlation to cancer) was red module with Gene Ontology related to cyclic adenosine monophosphate (cAMP) and cAMP response element-binding protein (CREB) activity. We selected the novel lncRNA *RP11-109D20.2* as a central gene in this module and confirmed its aberrant expression in 46 fresh tissue samples using real-time PCR.

Our results revealed that the unannotated lncRNA, *RP11-109D20.2*, is one of the most important upregulated lncRNAs in CRC and its putative function in gene expression may be through cAMP/CREB signaling pathway. Taken together, *RP11-109D20.2* can be considered as a novel diagnostic biomarker for early detection of CRC, although further research is required to confirm this.

A Novel Homozygous Variant in Homologous Recombination Repair Gene *ZSWIM7* Causes Azoospermia in Males and Primary Ovarian Insufficiency in Females

Shah Hussain[a†], **Shoaib Nawaz**[b,c†*], Ihsan Khan[d], Nida Khan[e], Shabir Hussain[a], Imran Ullah[a], Khalid A. Fakhro[c,f,g], Wasim Ahmad[a#]

Affiliations:

[a] Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan

[b] Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan.

[c] Department of Human Genetics, Sidra Medicine, Doha, Qatar.

[d] First Affiliated Hospital of USTC, Hefei National Laboratory for Physical Sciences at Microscale, the CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Basic Medical Sciences, Division of Life Sciences and Medicine, CAS Center for Excellence in Molecular Cell Science, Collaborative Innovation Center of Genetics and Development, University of Science and Technology of China, Hefei 230027, China.

[e] Department of Obstetrics & Gynecology, Hayatabad Medical Complex, Peshawar, Pakistan.

[f] Department of Genetic Medicine, Weill Cornell Medical College, Doha, Qatar.

[g] College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar.

[†] Contributed Equally

*Presenting Author

#Corresponding Author

ABSTRACT

Infertility is a common, clinically heterogeneous reproductive disorder worldwide with a prevalence of about 15%. To date about 80 genes have been discovered to cause non-syndromic infertility, affecting males and females equally, though traditionally the genetic analysis of each group has been conducted separately. Here, we report the clinical and genetic characterization of a consanguineous family of Pakistani origin with multiple individuals, including male and female, affected with infertility. Males exhibited azoospermia whereas females had primary ovarian insufficiency.

Whole exome sequencing revealed a missense variant (c.176C>T, p.(Ser59Leu)) in the *ZSWIM7* gene which functions in homologous recombination repair. The variant was found in a homozygous form in all affected males and females. To our knowledge, this is the first mutation in *ZSWIM7* to be shown to cause infertility in both sexes, pointing to the utility of large consanguineous families with multiple affected siblings to reveal joint mechanisms affecting human reproduction.

Keywords

Infertility, azoospermia, primary ovarian insufficiency, *ZSWIM7*

Application of Receptor binding domain bioinformatics to glucokinase – an orthogonal approach to predict functional change from genetic mutation

Maryam Al-Motawa* and Paul J. Thornalley#
Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar

The aim of this study is to apply bioinformatics “Receptor Binding Domain” (RBD) analysis to predict functional change of glucokinase (GCK) from genetic mutation. GCK is low affinity hexokinase-4 which functions as a glucose sensor for pancreatic beta-cell insulin secretion and hepatic glucose metabolism. Loss-of-function mutations in GCK produce maturity-onset diabetes of the young, type 2 (MODY2), characterized in heterozygotes by stable mild fasting hyperglycemia. It is one of the most common forms of monogenic diabetes which currently may be misdiagnosed. There are also rare gain-of-function mutations associated with hyperinsulinemic hypoglycemia. Currently, the effect of non-synonymous single nucleotide polymorphisms of GCK on phenotype is predicted by “Sorting Tolerant From Intolerant” (SIFT) or similar algorithm which assumes highly conserved sequence positions are important and change of these may affect protein function. It provides no information on type of functional change.

RBD analysis is a sequence-based method to predict amino acid location in functional domains: receptor-binding domain – sites of protein-protein, protein-ligand and enzyme-substrate interaction, surface domain (S), interior of globular domain (G) and membrane domain (M) from a plot of mean hydrophobic moment versus mean Eisenberg hydrophobicity for a window of 5 amino acids along the sequence. When RBD analysis for a mutation predicts movement of one or more residues out of the RBD, a loss-of-function is expected; and when it predicts one or more residues move into the RBD, a gain-of-function mutations is expected. Examples are: mutation R397L, predicting residues 395, 298 and 399 move out of the RBD – a known inhibitory mutation of MODY2; and mutation W99R, predicting movement of residues 97, 98 and 100 into the RBD – a known activating mutation.

With further refinement, RBD analysis may provide an additional orthogonal approach to SIFT-like algorithms to improve diagnosis of MODY2 mutations and access to appropriate clinical treatment and care.

Theme: Big data, new technologies, and translational genomics

The Triglyceride Glucose-Waist to Height ratio outperforms obesity and other triglyceride-related parameters in detecting prediabetes in normal-weight adults in Qatar: a cross-sectional study

Neyla S. Al Akl[1], Elias N. Haoudi[2#], Halima Bensmail[3] and Abdelilah Arredouani[1*]

[1]Diabetes Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar.
[2]Qatar Academy, Qatar Foundation, Doha, Qatar.
[3]Qatar Center for Artificial Intelligence (QCAI), Qatar Computing Research Institute (QCRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar

***Correspondence:** Abdelilah Arredouani: aarredouani@hbku.edu.qa
#Presenting author: Elias N. Haoudi: elias2005h@gmail.com

Background: The triglyceride-glucose (TyG) driven parameters incorporating obesity indices have been proposed as reliable markers of insulin resistance and related comorbidities such as diabetes. This study evaluated the effectiveness of these indices in predicting prediabetes in healthy weight individuals from the middle east.
Methods: Using the data of 5996 Qatari participants from Qatar BioBank with machine learning algorithms, we analyzed the predictive value of different triglyceride and obesity indices in prediabetes prevalence in healthy weight individuals defined by body mass index between 18.5-24.9 kg/m^2 and are above 18 years old.
Results: Of the healthy-weight adults, 13.62% had prediabetes. TyG waist to height ratio (WHTR) was significantly associated with prediabetes among healthy weight men and women, respectively, (odds ratio OR per SD 2.68; 95% CI 1.67–4.32) and (OR per SD 2.82; 95% CI 1.61-4.94). Compared with other indices, TyG-WHTR had the highest area under the curve (AUC) value for prediabetes in men (AUC: 0.76, 95% CI 0.70–0.81) and women (AUC: 0.73, 95% CI 0.66–0.80) and performed significantly higher than other indices $p<0.05$ in predicting prediabetes in men. Tyg-WHTR shared a similar predictive effect as fasting plasma glucose (FPG)
Conclusion: Our study demonstrated that the TyG- WHTR index is a superior indicator to predict prediabetes in healthy weight men and is so conveniently accessible; as such, the TyG-WHTR index may be considered for clinical practice as a potential indicator for predicting the incidence of prediabetes in healthy weight men in Qatar.

Studies of a knockout mouse model supports functional role and potential therapeutic target for heat shock protein DNAJB3 in risk of obesity

Shadi Nejat[a], Kalhara Menikdiwela[a], Shane Scoggin[a], Israa Elbashir[b]*, Mohammed Dehbi[b], Paul J Thornalley[b#], and Naima Moustaid-Moussa[a]
[a]Department of Nutritional Sciences, College of Human Sciences and Obesity Research Institute, Texas Tech University and [b]Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar

The aim of this study was to assess if gene deletion of heat shock protein DNAJB3 exacerbates the obesity phenotype in the high fat diet (HFD) fed mouse model of obesity. Obesity and related metabolic dysfunctions are partly mediated by chronic low-grade inflammation and altered expression of numerous proteins, including heat shock proteins (HSPs). In previous studies, low expression of DNAJB3 was found in muscle of patients with obesity and type 2 diabetes (T2D). This was partly restored by increased physical activity. We generated DNAJB3 knockout (KO) mice and evaluated the obesogenic response to HFD feeding, compared to wild type (WT) control littermates.

DNAJB3 KO mice were generated using the CRISPR/Cas 9 approach. Male and female KO and WT mice were fed HFD (45 kcal% fat) or low fat diet (LFD; 10 kcal% fat) for 12 weeks. Body weight and food intake were measured weekly. Body composition and glucose tolerance tests (GTTs) were also assessed during the intervention. Following euthanasia, blood, and tissues were harvested. Serum was analyzed for adipokines and hormones using multiplex immunoassays.

HFD-fed KO mice had higher body weight and fat mass, compared to WT male and female controls. Both male and female KO HFD groups demonstrated lower glucose clearance rates in GTTs, compared to LFD KO and LFD and HFD WT groups. These effects were greater in female HFD KO groups which had higher serum leptin, interleukin-6 and insulin levels, compared to all other groups ($p<0.0001$). Leptin and resistin levels were higher male HFD KO mice, compared to all other groups ($p=0.0001$, $p=0.0253$).

We conclude that lack of DNAJB3 increases adiposity and glucose intolerance in diet-induced obese male and female mice. DNAJB3 may have a role in metabolic regulation and glucose homeostasis and may be a potential therapeutic target for the prevention of obesity and T2D.

Theme: Animal, cellular, and organoid models for human disease modeling

ABSTRACT

High Glucose Concentration-Induced Dicarbonyl Stress in Human Aortal Endothelial Cells Activates the Unfolded Protein Response and NLRP3 Inflammasome

Mingzhan Xue[a]*, Naila Rabbani[b] and Paul J. Thornalley[a#]

Affiliations: [a]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar; and [b]Department of Basic Medical Science, College of Medicine, QU Health, Qatar University, Doha, Qatar.

The aim of this study was to investigate the role of hyperglycemia-induced dicarbonyl stress in activation of the unfolded protein response (UPR) and NLRP3 inflammasome using the *in vitro* model of human aortal endothelial cells (HAECs) in high glucose concentration cultures. Metabolic dysfunction of endothelial cells in hyperglycemia contributes to the development of vascular complications of diabetes in which the NLRP3 inflammasome is involved. The mechanism is unclear. High glucose concentration leads to increased formation and accumulation of reactive metabolite, methylglyoxal (MG) - called dicarbonyl stress. MG modifies proteins producing physiological activators of the UPR. MG is metabolized by glyoxalase 1 (Glo1). We investigated the involvement of dicarbonyl stress in activation of UPR and NLRP3 inflammasome by siRNA silencing of Glo1.

HAECs were cultured in low glucose (4.1 mM, LG) and high glucose (20 mM, HG) concentration for 72 h, with and without prior treatment with 50 nM Glo1 siRNA (Glo1KD). Protein abundance of UPR pathway mediators, XBP1, PERK and ATF6, and NLRP3 mediator, thioredoxin interacting proteins (TXNIP), were assessed. Data are mean ± SD (n = 3).

In assessment of UPR activation, XBP1 protein was increased 43 ± 16% by Glo1KD in LG ($P<0.05$), 68 ± 3% in HG ($P<0.01$) and 153 ± 21% by Glo1KD in HG ($P<0.001$). PERK protein was decreased 58 ± 4% by Glo1KD in LG ($P<0.05$), 21 ± 2% in HG ($P<0.01$) and decreased 63 ± 2% by Glo1KD in HG ($P<0.001$). ATF6 protein was unchanged. TXNIP was increased 54 ± 21% by Glo1KD in LG ($P<0.05$), 112 ± 12% in HG and 159 ± 9% by Glo1KD in HG ($P<0.001$).

We conclude that dicarbonyl stress induced in aortal endothelial cells in high glucose concentration activates both the UPR and NLRP3 inflammasome. These responses likely contribute to vascular inflammation and endothelial dysfunction in diabetes.

Theme: Animal, cellular, and organoid models for human disease modeling

Overexpression of glyoxalase 1 is a negative survival factor for a wide range of human tumors – a target for innovative Glo1 inhibitor therapy

Naila Rabbani[a]* and Paul J. Thornalley[b#]

[a]Department of Basic Medical Science, College of Medicine, QU Health, Qatar University, Doha, Qatar and [b]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar

The aim of this study is to assess the association of expression of glyoxalase 1 (Glo1) with cancer patient survival using on-line databases. Glo1 catalyses the metabolism of the reactive, cytotoxic dicarbonyl metabolite, methylglyoxal (MG), which is formed mainly as a by-product in anaerobic glycolysis. Human tumors often have high rates of MG formation linked to high glycolytic rates and have related high Glo1 expression protective of the tumor and permissive of rapid tumor growth. Many clinical anticancer drugs increase tumor MG as part of their cytotoxic mechanism of action. High Glo1 expression may thereby be a mediator of multidrug resistance and biomarker of rapid tumor growth which may be countered by Glo1 inhibitor therapeutics.

We investigated the association of tumor Glo1 expression with survival in the Kaplan-Meier cancer patient-gene expression database (http://kmplot.com/analysis/) independent of tumor stage, genotype and treatment. Hazard ratio (HR) and logrank P for survival analysis are given (n = patient number). Glo1 expression was determined by RNAseq and patient survival assessed as overall survival or progression-free survival.

High Glo1 expression is a negative survival factor for prognosis of human tumors. Median survival, high vs low Glo1 expression: liver - 11.6 vs 29.7 months, HR = 1.67 ($P<0.001$, n = 370); ovarian - 42.1 vs 50.3 months, HR = 1.33 ($P = 3.1 \times 10^{-5}$, n = 1656); breast – 102 vs 142 months, HR = 1.41 ($P<0.05$, n = 1089); uterine – 42 vs 108 months, HR = 1.72 ($P<0.01$, n = 542); sarcoma – 37.2 vs 85.8 months, HR = 2.54 ($P = 4 \times 10^{-6}$, n = 259); and pancreatic - 17.3 vs 22.0 months, HR = 1.59 ($P = 0.026$, n = 177).

Glo1 inhibitor treatment has proven effective in tumor-bearing mice – evaluated against lung, prostate and glioblastoma multiforme tumors. It is innovative therapy waiting clinical evaluation.

Theme: Advances in Innovative Therapies for Precision Medicine

Saliva is a Source of biomarkers to predict diabetes at an early stage

Selvasankar Murugesan[a], Mohammed Elanbari[b], Annalisa Terranegra[a], Souhaila Al Khodor[a*]
[a] Maternal and Child Health Program, Sidra Medicine, Doha, Qatar
[b] Clinical Research Center Department, Sidra Medicine, Doha, Qatar
* - Corresponding author

Introduction
Type 2 diabetes (T2D) is a global health problem affecting more than 462 million people. Qatar Biobank (QBB) reported a worsening situation, with 13.9% of adults aged 18-64 suffering from T2D. However, the role of the salivary microbiome (SM) in T2D pathogenesis remains unclear. Our study aims to study the SM composition in Qatari subjects with or without T2D and identify microbial signatures associated with T2D.

Methods
Saliva samples collected from 2974 QGP participants were retrieved from QBB. Based on the HbA1C level and treatment-related questionnaire, all participants were classified into non-diabetic (HbA1C<5.7) (n=2166), pre-diabetic (HbA1C=5.7-6.5) (n=349) and diabetic (>6.5) (n=459). 16S-rDNA libraries were sequenced and analyzed using the QIIME pipeline to assess the SM composition. Machine Learning (ML) strategies were used to identify SM-based predictors of T2D.

Results
Firmicutes and Bacteroidetes were the predominant phyla among all Qatari participants. *Pseudomonas, Acinetobacter, Micrococcus, Rhodococcus, Comamonas,* and Chryseobacterium are the significantly abundant genera in the non-diabetic group; *Enhydrobacter, Cloacibacterium, Bilophila, Acholeplasma,* and *Azospirillum* were greatly enriched in the diabetic subjects, while *Exiguobacterium, Odoribacter, Alistipes* were significantly abundant in the pre-diabetic groups. ML-based prediction models revealed that *Anaerotruncus* and *Enterobacteriaceae* were the common predictors of T2D.

Conclusions
This work is the first study to assess SM using T2D as a disease model in a large cohort of the Qatari population. In addition, this study affirms the specific microbial signature changes in diabetic and non-diabetic subjects. Integrating multi-omics data such as metagenomics, meta-transcriptomics, metaproteomics, and metabolomics will reveal potential predictive biomarkers for T2D.

Acknowledgment
Data and samples were obtained from QBB (www.qatarbiobank.org.qa). This project was financially supported by QNRF- PPM2-0216-170012 and Qatar genome to Dr. Souhaila Al Khodor.

The entero-mammary microbiota during a SARS-CoV-2 context

Juan Manuel Vélez-Ixta[1*], Carmen Josefina Juárez-Castelán[1], Karina Corona-Cervantes[1], Alberto Piña-Escobedo[1], Yair Cruz-Narváez[2], Alejandro Hinojosa-Velasco[3], María Esther Landero-Montes de Oca[4], Eduardo Dávila-Gonzalez[5], Eduardo González-del Olmo[6], Fernando Guadalupe Bastida-González[5], Paola Berenice Zárate-Segura[6], Jaime García-Mena[1#]

[1]Departamento de Genética y Biología Molecular, Cinvestav, Av. IPN 2508, Ciudad de México, México.
[2]Laboratorio de Posgrado de Operaciones Unitarias, Escuela Superior de Ingeniería Química e Industrias Extractivas, Instituto Politécnico. Ciudad de México, Mexico.
[3]División de Neonatología, Hospital de Ginecología y Obstetricia, Instituto Materno Infantil del Estado de México, Estado de México, Mexico.
[4]Departamento de Ginecología y Obstetricia, Hospital Gustavo Baz Prada, ISEM, Estado de México, México.
[5]Laboratorio Estatal de Salud Pública del Estado de México, ISEM, Toluca de Lerdo, Estado de México, México.
[6]Laboratorio de Medicina Traslacional, Escuela Superior de Medicina, Instituto Politécnico Nacional, Ciudad de México, México.
Corresponde: [*]juanvelez96@outlook.com, [#]jgmena@cinvestav.mx

COVID-19 is a severe respiratory disease threatening pregnant women, which increases the possibility of adverse pregnancy outcomes. Several recent studies have demonstrated the ability of SARS-CoV-2 to infect the mother enterocytes disturbing the gut microbiota diversity. The aim of this study was to characterize the entero-mammary microbiota of women in presence of the virus during delivery. Fifty mother-neonate pairs were included in a transversal descriptive work. The presence of SARS-CoV-2 RNA was detected in nasopharyngeal and rectal swabs (RS) collected from pairs, and human colostrum (HC) samples collected from mothers. The microbiota diversity was characterized by high-throughput DNA sequencing of V3-16S rRNA gene libraries prepared from HC and RS. Data was analyzed with QIIME2 and R. Our results indicate that several bacteria taxa are highly abundant in mother RS positive to SARS-CoV-2 RNA. These bacteria mostly belong to the Firmicutes phylum; for instance, *Megasphera*, *Ruminococcus* and *Akkermansia* (Verrucomicrobiota Phylum) have been previously associated with anti-inflammatory effects which could explain the capability of women to overcome the infection. HC featured a high abundance of the Firmicutes phylum, both in positive and negative samples for the virus. Further data analysis showed that nearly 20% of the bacterial diversity found in HC was also identified in mother's RS. Spearman correlation analysis highlights that some genera of the Proteobacteria and Actinobacteria phyla are negatively correlated with nasopharyngeal swab ($p < 0.005$). This study provides new insights into the gut microbiota of pregnant women and their potential association with a better outcome during SARS-CoV-2 infection.

Mutation of *TRPC6*, autism candidate gene, induces hyperexcitability of neurons by reducing store-operated calcium entry (SOCE)

Kyung Chul Shin[1*], Gowher Ali[1*], Houda Yasmine Ali Moussa[1], Lawrence W Stanton[1,2†], **Yongsoo Park**[1,2,3†]

[1]Neurological Disorders Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[2]College of Health & Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[3]The presenting author

*These authors contributed equally to this work.
†Corresponding authors;

Abstract

Background: Autism spectrum disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder, mainly caused by rare and de novo genetic variants and mutations. ASD co-occurs with comorbid attention-deficit hyperactivity disorder (ADHD) and epilepsy, which are associated with hyperexcitability of neurons. Transient receptor potential family C-6 (TRPC6) is a candidate risk factor for ASD and implicated in ASD etiology; de novo missense and nonsense mutations in TRPC6 associated with ASD etiology have been reported.

Objectives: Loss-of-function mutations in TRPC6 reduce calcium influx in human induced pluripotent stem cell (hiPSC)-derived neurons cell and TRPC6 knockdown (KD) in *Drosophila* causes autism-like behavioral deficits and leads to the hyperactivity phenotype. However, the pathophysiology underlying hyperactivity phenotype caused by TRPC6 mutations in ASD is unclear and mains poorly understood. Here we aim to study the pathophysiological mechanisms underlying ASD hyperactive phenotype with focus on calcium signaling.

Methods: The major impediment to ASD research is to produce relevant animal and cellular models. To overcome this limitation, we have generated hiPSC-derived cortical neurons as a platform to model pathology of ASD, and performed single cell calcium imaging and electrophysiological recording to investigate the pathophysiological mechanisms underlying ASD hyperactive phenotype.

Results: We observed that TRPC6 knockout (KO) in hiPSC-derived cortical neurons reduces store-operated Ca^{2+} entry (SOCE) signaling and leads to hyperexcitability of neurons by increasing action potential frequency and network burst frequency. TRPC6 KO increased the length and numbers of neurites, and numbers of branches in neurites, implying that hyperactivity

of synchrony and neural network might be induced by increasing the number of synaptic connections.

Significance: Our data show the molecular and cellular pathophysiology underlying hyperactivity of ASD individuals, and TRPC6 KO hiPSC-derived cortical neurons can be a good model to study hyperactive behavior of ASD and will be used to screen therapeutics that reverse hyperexcitability.

HNF4α Could be the Missing Link between EMT and Warburg Effect during Hepatocarcinogenesis

Bahare Shokoohian[1, 2], Babak Negahdari[1], Zahra Heydari[2], Mehdi Totonchi[3], Hamidreza Aboulkheyr Es[4], Abbas Piryaei[5, 6], Massoud Vosough[2, 7]*

1- Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

2- Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

3- Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

4- School of Biomedical Engineering, University of Technology Sydney, Sydney, Australia.

5- Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran 16123798, Iran

6- Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran 16123798, Iran.

7- Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.

***Corresponding author:**

Massoud Vosough, MD, Ph.D. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.

Email address: massoud.vosough@ki.se

ABSTRACT

Hepatocellular carcinoma (HCC) is a heterogeneous, late-diagnosed, and highly recurrent malignancy that often affects the whole body's metabolism. Therefore, finding certain theranostic molecules which can address current concerns simultaneously is one of the priorities in dealing with the HCC. In this study, performing protein-protein interaction network analysis proposed HNF4α as a hub protein, associating epithelial-mesenchymal transition (EMT) to reprogrammed cancer metabolism, formerly known as the Warburg effect. Both phenomena improved the compensation of cancerous cells in competitive conditions. Mounting evidence has demonstrated that HNF4α is commonly down-regulated and serves as a tumor suppressor in the HCC. Enhancing the HNF4α mRNA translation rate through a specific synthetic antisense long non-coding RNA, profoundly affects both EMT and onco-metabolic modules in HCC cells. HNF4α over-expression decreased featured mesenchymal transcription factors and improved hepatocytic function, as well as glycolysis deceleration, accelerated gluconeogenesis, and improved dysregulated cholesterol metabolism. HNF4α over-expression, inhibited migration, invasion, and proliferation of HCC cells *in vitro* and decreased metastasis rate and tumor growth in xenografted nude mice model. Our findings suggest a central regulatory role for HNF4α, through its broad access to a wide variety of gene promoters involved in EMT and the Warburg effect in human hepatocytes. This essential impact indicates that HNF4α may be a potential target for HCC treatment.

Keywords: Hepatocellular Carcinoma, Aerobic Glycolysis, Warburg effect, Epithelial-Mesenchymal Transition, HNF4α over-expression

Investigating the DNA methylation profile of childhood solid tumors to identify a novel diagnostic panel

Sarah A. Albasha[a*], Abdulmonem A. Alsaleh[a#], Noura N. Alibrahim[a], Aymen Al Qurain[b], Bader Al Alwan[a], Yahya Aldawood[a], Nader A. Mohammed[a], Fardus A. Aljunibi[a], Mohammad Al Khamees[c], Fatimah J. Al Shaikh[d], Hibbah H. Alshuwaikhat[e], Jenan Almatouq[a]

[a]*Department of Clinical Laboratory Science, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[b]*Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[c]*Department of Clinical Laboratory, King Fahad Hospital in Hofuf, Al Hasa, Saudi Arabia*
[d]*Department of Physical Therapy, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[e]*Department of Respiratory Care, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
**Presenting author; # Corresponding author*

Abstract:

Background: Solid tumors in children represent approximatively 30% of all pediatric cancers. The most prevalent types involve Wilms' tumor, retinoblastoma, neuroblastoma and Ewing's sarcoma. The literature suggests a degree of similarity between these conditions (such as age of diagnosis, prognosis, etc), suggesting that they may share a similar pathogenesis. This study aims to investigate the DNA methylation profile of common childhood solid tumors to identify a common signature that can be used to understand the underlying common molecular mechanism. **Method:** This is an in-silico meta-analysis study which used publicly available data from a DNA methylation array. Illumina 450K array datasets of the four conditions were downloaded from the GEO data repository and processed on RStudio using ABC.RAP R package. Differentially methylated sites were selected based on scoring Student's t-test *P*-value ≤ 0.001 (FDR); and a methylation difference (delta beta) ≥ 20%. Differentially methylated genes were defined as genes having at least two differentially methylated sites according to the human GRCh37/hg19 annotation. **Results:** The analysis revealed 17 novel differentially methylated genes that remarkably share a common methylation profile between the four tumors. Extended comparison analysis with childhood acute lymphoblastic leukaemia showed consistent methylation results for the identified candidate genes. **Conclusion:** The novel discovery of the 17 common differentially methylated genes among four common childhood solid tumors provide an insight towards understanding their shared molecular pathogenesis. In addition, the identified candidate genes can be used as biomarkers for the clinical diagnosis of childhood solid tumors.

Flow-Cytometry evaluation of Cellular Surface FVIII in Blood cells and its correlation with FVIII activity and coagulation

Anjud Al-Mohannadi[1*], Muhammad Elnaggar[4], Hani Bibawi[2], Igor Pavlovski[1], Giusy Gentilcore[1], Mohammed J Al-Kubaisi[1], Che-Ann Lachica[1], Dhanya Kizhakayil[1], Mohammed Elanbari[1], Elkhansa Elgaali[3], Ayman Saleh[3], Chiara Cugno[1,3], Sara Deola[1#]

[1] Research Department, Sidra Medicine, PO Box 26999, Doha, Qatar.
[2] Pathology Department, Sidra Medicine, PO Box 26999, Doha, Qatar.
[3] Hematology-Oncology Clinic, Sidra Medicine, PO Box 26999, Doha, Qatar.
[4] Icahn School of Medicine at Mount Sinai, Division of Hematology and Medical Oncology, Hess Center, New York, NY 10029

FVIII deficiency or inactivity is known as Hemophilia A. On the contrary, FVIII overactivity is linked to hypercoagulability state. In steady state, FVIII remains covalently bound to vWF, and upon coagulation cascade activation, active FVIII (FVIIIa) binds to the plasma membrane of platelets and other blood cells via its C2 domain to exert its coagulative role. Recently, we developed a reliable Flow-Cytometry platform for FVIII detection suitable for intracellular and extracellular FVIII measurement (Elnaggar M. et al, 2020). Leukocyte abundance is reported to be associated with thrombotic events but without clear mechanistic explanation. Therefore, we sought to evaluate the percentage of FVIIIa bound on leukocyte subsets (lymphocytes, monocytes, and granulocytes) in different clinical scenarios, including HA and other abnormal coagulation states and to correlate it to FVIII plasmatic levels. The analysis was conducted on 31 pediatric subjects (median age 9y), including patients with: HA (n=8), coagulopathies (von Willebrand Disease, menorrhagia, and unexplained bleedings (n=11) and 1 patient with known high FVIII levels), and healthy controls (n=11). We performed complete blood count on fresh blood samples as well as in-house-developed flow-cytometry. In parallel, plasma-FVIII measurements were performed fand results were correlated FC data. Expectedly, HA-patients showed the lowest plasma-FVIII ($p<0.0001$), while leukocytes' surface FVIII was generally low (on average <1%), trended to a lower rate in patients with coagulopathies and HA compared to healthy-controls and was significantly higher in the patient with hypercoagulability. Surprisingly, among leukocytes' subpopulations, screened on patients with bleeding diathesis and HA subjects *vs* healthy-controls, only CD8-surface bound FVIIIa was significantly lower compared to healthy-controls ($p=0.015$ healthy-controls vs HA, and $p=0,0093$ healthy-controls vs bleeding diathesis). These results indicate a role of CD8-cell mediated FVIII binding mechanism regulating its availability in case of biological need as bleeding. We did not find any significant correlation between leukocyte-surface bound FVIIIa and plasma FVIII level, indicating that the differences in leukocyte surface bound FVIIIa are not directly proportional to FVIII levels in plasma, rather actively regulated by unknown mechanism. While the study is still ongoing, we showed in this preliminary analysis that our in-house FC platform for FVIII detection screens in depth FVIII adherence to blood cells, may shed light on coagulation novel mechanisms, and potentially serve as a diagnostic and prognostic tool.

Appendix

Table I

Patient Group	Age	Gender	Reason for coming to clinic	FVIII plasma level	FVIII% on leukocyte surface
Hypercoagulative	4 yrs	F	High coagulation level	>150	0,83
No coagulopathies	31yr1mo	F	Healthy adult	112	0
No coagulopathies	17 yrs	F	Gastritis	95	0,018
No coagulopathies	3 mo	M	Meningitis	83	0,18
No coagulopathies	2yrs7mo	F	Infantile Osteopetrosis post BMT	>150	0,17
No coagulopathies	35 yrs	F	Missed miscarriage	124	0,3
No coagulopathies	12yr2 mo	M	Prematurity, spastic diplegic cerebral palsy ,rheumatic heart disease, obesity	75	0,142
No coagulopathies	11 yrs11 mo	F	Developmental and learning delay, bilateral sensorineural deafness, epilepsy, hypothyroidism	104	0,5
No coagulopathies	14yr6mo	M	Dandy Walker	>150	0,036
No coagulopathies	24yr8mo	F	Hypothyroidism gestational	126	0,158
No coagulopathies	5 mo	F	Unknown	105	0,01
No coagulopathies	10yrs4m	F	Bilateral choanal atresia, asthma, right ectopic kidney	75	0
Coagulopathies	14 yrs	F	Menorrhagia	104	0,46
Coagulopathies	11yrs4mo	F	Menorrhagia	53	**0,47**
Coagulopathies	9yr1mo	F	Epistaxis /Atopic dermatitis	96	0,042
Coagulopathies	13 yrs	F	DM type1/Menorrhagia	106	0,1
Coagulopathies	9yrs	F	Bleeding from nipple	81	0
Coagulopathies	17 yrs	F	Menorrhagia	>150	0,011
Coagulopathies	17 yrs	F	Menorrhagia	> 150	0,076
Coagulopathies	9 yrs	F	Menorrhagia	112	0,1
Coagulopathies	6 mo	M	Von Willebrand	31	0
Coagulopathies	5yrs	M	Von willebrand	150	0
Coagulopathies	15yrs	F	Menorrhagia	86	0,012
Hemophilia A	2yr7mo	M	Hemophilia A	10	0
Hemophilia A	6 wks	M	Moderate Hemophilia A	3	0,083
Hemophilia A	2yrs	M	Severe hemophilia A with inhibitors	7	0,04
Hemophilia A	23 mo	M	Severe Hemophilia A	<l	0,2
Hemophilia A	22 mo	M	Severe Hemophilia A	41	0,2
Hemophilia A	8yrs	M	Severe Hemophilia A	17	0,01
Hemophilia A	4yrs	M	Mild Hemophilia A	5	0,07
Hemophilia A	17 mo	H	Mild Hemophilia A	8	0,01

Legend. Patients-groups' characteristics, FVIII plasma levels and FVIII leukocytes surface % measured with Flow Cytometry. Each measurement shows the FVIII% after non-relevant IgG control % subtraction.

Identification of common differentially methylated genes among different lung cancer subtypes

Manar Z. Almotawa[a*], Abdulmonem A. Alsaleh[b#], Zaheda H. Radwan[b], Alaa K. Al Helaili[c], Zahiah F. Al Mohsen[c], Maryam Al Ramadan[b], Dunya Al Faraj[d], Silas Mangi[b], Hanan A. Alnajrani[a], Wesam A. Almgabi[b], Yousef N. Alhashem[b], Jenan Almatouq[b]

[a]*Department of Respiratory Care, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[b]*Department of Clinical Laboratory Science, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[c]*Department of Foundation Year, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia*
[d]*Department of Emergency Department, King Fahad University Hospital, Dammam 34222, Saudi Arabia*
**Presenting author; # Corresponding author*

Abstract:

Background: Lung cancer is one of the leading cancers worldwide. Histologically, it is classified into two types: small cell and non-small cell lung cancers. The non-small cell lung cancer is further subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. The literature suggests a unique DNA methylation signature for each cancer type, however, there is no consensus on a common panel for all of the lung cancer classes. This study aims to identify a common methylation profile for all types of lung cancer that can be used potentially as a screening or a diagnostic panel. **Method:** Publicly available DNA methylation array data (Illumina 450k array and Illumina EPIC array datasets) for all lung cancer types were used for the data analysis. Datasets were analyzed on RStudio using ABC.RAP R package. Differentially methylated sites were selected based on scoring Student's t-test *P*-value $\leq 1 \times 10^{-7}$ (Bonferroni adjustment); and a methylation difference (delta beta) $\geq 30\%$. Differentially methylated genes were defined as genes having at least two differentially methylated sites according to the human GRCh37/hg19 annotation. **Results:** A total of 183 cases and 85 controls from 6 different datasets were analyzed. The analysis revealed 30 differentially methylated genes, from which 4 novel genes, showing a consistent methylation pattern among the four types of lung cancer. Further gene pathway analysis will be carried out to identify the underlying common mechanism. **Conclusion:** The identification of differentially methylated genes that have common pattern among all lung cancer types has a great clinical significance. The utility of that gene panel for the early clinical detection or screening may help early diagnosis, early prevention or at least improving patient's prognosis and quality of life.

Identifying novel interactions of the colon-cancer related APC protein with Wnt-pathway nuclear transcription factors

Nayra M. Al-Thani[1,3], Stephanie Schaefer-Ramadan[1], Jovana Aleksic[1], Yasmin A. Mohamoud[2], Joel A. Malek[1,2*]

[1]Department of Genetic Medicine, Weill Cornell Medicine in Qatar, [2]Genomics Core, Weill Cornell Medicine in Qatar, [3]College of Health and Life Sciences, Hamad Bin Khalifa University

Abstract

Colon cancer is often driven by mutations of the adenomatous polyposis coli (APC) gene, an essential tumor suppressor gene of the Wnt β-catenin signaling pathway. APC and its interactions in the cytoplasm have been well studied, however various groups have also observed its presence in the nucleus. Identifying novel interactions of APC in the Wnt pathway will provide an opportunity to better understand the nuclear role of APC and ultimately identify potential cancer treatment targets. We used the all-vs-all sequencing (AVA-Seq) method to interrogate the interactome of protein fragments spanning most of the 60 Wnt β-catenin pathway proteins. Using protein fragments identified the interacting regions between the proteins with more resolution than a full-length protein approach. Pull-down assays were used to validate a subset of these interactions. 74 known and 703 novel Wnt β-catenin pathway protein-protein interactions were recovered in this study. There were 8 known and 31 novel APC protein-protein interactions. Novel interactions of APC and nuclear transcription factors TCF7, JUN, FOSL1, and SOX17 were particularly interesting and confirmed in validation assays. Based on our findings of novel interactions between APC and transcription factors and previous evidence of APC localizing to the nucleus, we suggest APC may compete and repress CTNNB1. This would occur through the binding of the transcription factors (JUN, FOSL1, TCF7) to regulate the Wnt signaling pathway including through enhanced marking of CTNNB1 for degradation in the nucleus by APC binding with SOX17. Additional novel Wnt β-catenin pathway protein-protein interactions from this study could lead researchers to novel drug designs for cancer.

Circulating noncoding RNAs as a Signature of Autism Spectrum Disorder Symptomatology

Salam Salloum-Asfar[1], Khaoula Errafii[1], Saba F. Elhag[1], Noothan J. Satheesh[1], Mohamed M. Emara[2] and Sara A. Abdulla[1*#]

[1]Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.
[2]Qatar University, Doha, Qatar

Presenting Author*, Corresponding Author[#]

Background: Autism spectrum disorder (ASD) has been defined to entail impairments in cognition and social communication, as well as the presence of repetitive behaviors and restricted interests. While heavily used, and to a degree useful, current diagnostic approaches rely heavily on subjective and behavioral components to define "neurotypical" and "autistic" individuals and are very much dependent on the expertise of the examiner. This leaves room for misdiagnosis, and an urgent need for a chemical test to provide concrete and measurable evidences of ASD.

Objective: This study aims on improving current techniques, identifying and stratifying ASD through a data-driven/ objective approach, by defining a circulating non-coding RNA profile, and utilizing it as a diagnostic biomarker of ASD.

Methods: We subdivided our DSM5 and ADOS assessed cohort (N=45) into two groups, the first of which entails children who express severe ASD symptoms and the second mild ASD symptoms. Using next-generation sequencing, we were able to identify several circulating noncoding RNAs (cir-ncRNAs) in plasma. Differential expression analysis was performed using the CLC Genomics Workbench 20.0.4 on miRNAs, piRNAs, snoRNAs, Y-RNAs, tRNAs, and lncRNAs between the two ASD groups.

Results: Our findings show expression stability of our targeted cir-ncRNAs in plasma. Furthermore, there were distinct and apparent expression profiles of specific cir-ncRNAs between the severe and mild groups. The results lead to a US provisional patent, which is currently being converted to a PCT application, to allow for further development (3 years invention protection), to mature the findings into a transcriptomic based assay for clinical diagnosis.

Conclusions: Our data highlights cir-ncRNAs as a promising, objective, biomarker for diagnosing ASD. More importantly, the clear distinction of biological expression between mild and severe cases, provides the capacity to better understand and stratify individuals within the spectrum. Furthermore, it allows for targeted therapeutic approaches.

Circulating noncoding RNAs as a Signature of Autism Spectrum Disorder Symptomatology

Salam Salloum-Asfar[1], Khaoula Errafii[1], Saba F. Elhag[1], Noothan J. Satheesh[1], Mohamed M. Emara[2] and Sara A. Abdulla[1*#]

[1]Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.
[2]Qatar University, Doha, Qatar

Presenting Author*, Corresponding Author[#]

Background: Autism spectrum disorder (ASD) has been defined to entail impairments in cognition and social communication, as well as the presence of repetitive behaviors and restricted interests. While heavily used, and to a degree useful, current diagnostic approaches rely heavily on subjective and behavioral components to define "neurotypical" and "autistic" individuals and are very much dependent on the expertise of the examiner. This leaves room for misdiagnosis, and an urgent need for a chemical test to provide concrete and measurable evidences of ASD.

Objective: This study aims on improving current techniques, identifying and stratifying ASD through a data-driven/ objective approach, by defining a circulating non-coding RNA profile, and utilizing it as a diagnostic biomarker of ASD.

Methods: We subdivided our DSM5 and ADOS assessed cohort (N=45) into two groups, the first of which entails children who express severe ASD symptoms and the second mild ASD symptoms. Using next-generation sequencing, we were able to identify several circulating noncoding RNAs (cir-ncRNAs) in plasma. Differential expression analysis was performed using the CLC Genomics Workbench 20.0.4 on miRNAs, piRNAs, snoRNAs, Y-RNAs, tRNAs, and lncRNAs between the two ASD groups.

Results: Our findings show expression stability of our targeted cir-ncRNAs in plasma. Furthermore, there were distinct and apparent expression profiles of specific cir-ncRNAs between the severe and mild groups. The results lead to a US provisional patent, which is currently being converted to a PCT application, to allow for further development (3 years invention protection), to mature the findings into a transcriptomic based assay for clinical diagnosis.

Conclusions: Our data highlights cir-ncRNAs as a promising, objective, biomarker for diagnosing ASD. More importantly, the clear distinction of biological expression between mild and severe cases, provides the capacity to better understand and stratify individuals within the spectrum. Furthermore, it allows for targeted therapeutic approaches.

Computational identification of an immune-related 3-lncRNA signature with prognostic connotation in a multi-cancer setting.

Shimaa Sherif[1,2], Raghvendra Mall[3,4], Hossam Almeer[3], Adviti Naik[5], Abdulaziz Al Homaid[3], Remy Thomas[5], Jessica Roelands[2,6], Sathiya Narayanan[2], Mahmoud Gasim Mohamed[7], Shahinaz Bedri[8], Salha Bujassoum Al-Bader[9], Kulsoom Junejo[10], Davide Bedognetti[1,2], Wouter Hendrickx[1,2#], Julie Decock[1,5*#].

[1] College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), Doha, Qatar. [2] Cancer Department, Research Branch, Sidra Medicine, Doha, Qatar. [3] Qatar Computing Research Institute (QCRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), Doha, Qatar. [4] St. Jude Children's Research Hospital, Memphis, Tennessee, U.S.A. [5] Translational Cancer and Immunity Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), Doha, Qatar. [6] Department of Surgery, Leiden University Medical Center, Leiden, the Netherlands. [7] Women's Hospital, Hamad Medical Corporation, Doha, Qatar. [8] Weill Cornell Medicine-Qatar, Doha, Qatar. [9] National Center for Cancer Care and Research (NCCCR), Hamad General Hospital, Doha, Qatar. [10] General Surgery Department, Hamad General Hospital, Doha, Qatar

* presenting author, # corresponding author

Background: Immunotherapy rapidly changed the cancer treatment landscape with durable responses in multiple cancer types. However, its success remains limited to a subset of patients propelling the search for biomarkers that can predict who will most likely benefit from immunotherapy. As such, immune-related gene signatures with prognostic significance in one or few cancers have been identified, however, the area of non-coding RNAs remains largely unexplored.

Materials and Methods: We refined a computational method that enables the mapping of long non-coding RNAs (lncRNA) to a coding-non-coding (CNC) gene correlation network and the identification of immune related lncRNA signatures with prognostic connotation in solid cancers.

Results: Using the TCGA breast cancer dataset as a discovery cohort, tumors were classified as immune favorable or unfavorable, as per the immunologic constant of rejection (ICR) gene signature, and differentially expressed lncRNAs were defined. Subsequent mapping to the CNC network identified 127 proxy protein-coding genes with enrichment in immune-related diseases and functions. Next, we set out to identify sets of immune-related lncRNAs with prognostic significance in breast cancer and investigated whether these would hold true for other TCGA solid cancers. We identified two 20-lncRNA signatures with comparable prognostic significance as ICR in 6 cancers. Moreover, we were able to reduce these two signatures into a smaller signature of 3 common lncRNAs that retained prognostic connotation across 11 solid cancer types and showed superior performance in kidney renal papillary cell carcinoma, low-grade glioma and uterine corpus endometrial carcinoma compared to the ICR-signature.

Conclusions: Using an in-house refined computational method we identified an immune-related 3-lncRNA signature with prognostic value in breast cancer and 10 other solid cancer types. These findings indicate that this set of 3 lncRNAs holds potential as a minimal informative lncRNA biomarker signature for immune favorable tumors and warrants further development for clinical implementation.

Immunomodulation by Lactate dehydrogenase C indicates a potential new opportunity for combination therapy.

Adviti Naik[1*], Remy Thomas[1], Julie Decock[1,2#]

[1] *Translational Cancer and Immunity Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar*

[2] *College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar*

* presenting author, # corresponding author

Background: Lactate dehydrogenase C (LDHC) is a cancer testis antigen with a highly-tumor specific expression and unfavorable prognostic connotation. In recent work, we demonstrated that targeting LDHC improves treatment response to the DNA damage response drugs cisplatin and olaparib. Here, we explored whether LDHC may be a candidate target for combinatorial treatment with immunotherapy by modulating immunological signals in the tumor microenvironment.
Materials and methods: TCGA and METABRIC public breast cancer datasets were interrogated for *LDHC* expression and cytotoxic T lymphocyte infiltration. In vitro *LDHC* silencing models were used to analyze immune-related gene expression (RT2 Profiler array), cytokine protein secretion (ProteomeProfiler array) and immune checkpoint expression (flow cytometry). Finally, T cell activation following co-culture was determined by IFN-γ ELISpot.
Results: Transcriptomic analysis demonstrated that basal-like and Her2-enriched breast tumors feature a higher *LDHC* expression than luminal tumors, which was correlated with a poorer overall and disease-specific survival. Furthermore, high *LDHC* expression diminished the favorable prognostic effect of cytotoxic T lymphocyte infiltration in Her2-enriched (TIDE score=1.97, p=0.049) and triple negative breast tumors (TIDE score=0.466, p=0.642). Silencing of *LDHC* in breast cancer cell lines resulted in the dysregulation of multiple immune-related genes and secreted cytokines, promoting signaling pathways involved in granzyme-mediated cell death; T cell proliferation, activation and differentiation; cytolysis and interferon gamma production. In addition, *LDHC* silencing reduced the expression of the PD-L1 and Gal-9 immune checkpoint ligands, suggesting additional levels of immunomodulation. Concordantly, functional analysis confirmed that *LDHC* silencing enahnced T cell activation in a co-culture setting.
Conclusions: Our current findings suggest that targeting *LDHC* may have a dual anti-cancer benefit, impairing DNA damage repair and tumor cell survival while supporting a favorable tumor immune microenvironment. As such, LDHC-based therapy could potentially improve clinical outcome when used in combination with DNA damage response drugs or immunotherapy.

Targeting of Lactate dehydrogenase C in conjunction with DNA damage response drugs as a novel combinatorial treatment for breast cancer precision medicine

Adviti Naik[1*], Remy Thomas[1], Julie Decock[1,2#]

[1] *Translational Cancer and Immunity Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar*

[2] *College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar*

* presenting author, # corresponding author

Background: Dysregulation of the DNA damage response (DDR) is a key inducer of genomic instability that may be exploited to enhance sensitivity to cytotoxic drugs and DNA repair inhibitors. We previously reported that silencing of Lactate Dehydrogenase C (LDHC) perturbs DDR signaling in breast cancer cells, resulting in impaired long-term survival and sensitization to DDR-related drugs. LDHC features a highly tumor-specific expression with limited therapeutic off-target effects, hence, we sought to explore the mechanistic networks and key molecules that contribute to LDHC-associated DDR dysregulation.

Materials and Methods: Firstly, we used an RNA sequencing approach to explore transcriptome-wide alterations in LDHC-silenced breast cancer cells and secondly, mass spectrometry analysis to identify LDHC-interacting proteins.

Results: Differential expression and network analysis of LDHC-silenced cells identified 55 down- and 47 up-regulated genes that were enriched in pathways related to cell cycle checkpoint control, BRCA1-mediated DNA damage response and NF-kb signaling. Upstream regulator analysis revealed mTOR and CASP3 as potential regulators of the LDHC-associated transcriptome network. Further analysis of the mTOR pathway confirmed a downregulation in protein expression of the pro-tumorigenic molecules STAT1, STAT3 and SMAD3 following LDHC silencing. Similarly, we observed a decrease in protein expression of the STAT3 downstream anti-apoptotic molecule Bcl2 in LDHC-silenced cells. Additionally, LDHC immunoprecipitation in combination with mass spectrometry identified Heterogeneous nuclear ribonucleoprotein M (hnRNP M) that directly interacts with the mTOR complex protein Rictor as a putative LDHC-binding partner. Functional mTOR and STAT3 inhibitor studies are ongoing to assess the extent to which these pathways contribute to the LDHC-silencing cellular phenotype.

Conclusions: Our findings highlight functional networks and molecular mediators that may play a role in DNA damage response dysregulation and loss-of-survival following LDHC therapeutic targeting. This knowledge may further guide the development of LDHC combinatorial treatment approaches for breast cancer precision medicine.

Lack of FOXA2 dysregulates miRNAs targeting key genes involved in pancreatic islet development

Noura Aldous[a*], Ahmed K. Elsayed[b], Nehad M. Alajez[a,c], Essam M. Abdelalim[a,b#]

[a]*College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar Foundation.*

[b]*Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation.*

[c]*Translational Cancer and Immunity Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation.*

**Presenting author, #Corresponding author*

Abstract

Background and aim: FOXA2 is crucial for human endocrine development during pancreatic islet differentiation. However, whether miRNAs are regulated by FOXA2 or not is largely unknown. In this report, we aimed to capture the dysregulated miRNAs in the absence of FOXA2 and identify their pancreatic-specific target genes in iPSC-derived pancreatic progenitors (iPSC-PPs).

Materials and Methods: FOXA2 knockout (*FOXA2*$^{-/-}$) hiPSC lines were generated using CRISPR/Cas9. *FOXA2*$^{-/-}$hiPSCs and their isogenic controls were differentiated into PPs *in vitro* using our protocol. RNA-sequencing and miRNA-sequencing were performed for *FOXA2*$^{-/-}$-PPs and WT-PPs. Pathways analyses were performed on differentially expressed genes (DEGs), miRNAs, and miRNA target genes identification was performed using Ingenuity Pathway Analysis (IPA) software. RT-qPCR was performed for validation.

Results: In *FOXA2*$^{-/-}$-PPs, 1628 down-regulated (Log2 FC < −1.0, $p < 0.05$) and 769 up-regulated (Log2 FC > 1.0, $p < 0.05$) DEGs were found. Several important transcription factors (TFs) required for pancreatic islet development and function were significantly downregulated in *FOXA2*$^{-/-}$PPs such as *PDX1, NKX6.1, SOX9, NGN3*, *NEUROD1*, and *PTF1A*. In miRNA-seq analysis, 107 downregulated (Log2 FC < −1.0, $p < 0.05$) and 111 upregulated (Log2 FC > 1.0, $p < 0.05$) differentially expressed miRNAs were found. Integrating miRNA and mRNA profiling showed 190 miRNAs targeting 2654 mRNAs. Our analysis showed that upregulated miRNAs (miR-92a-2-5p, miR-92b-5p, and miR-184) targeted both *FOXA2* and *NKX6.1*, while miR-124-3p targeted multiple important pancreatic TFs including *FOXA2, NGN3, NEUROD1, GATA6, SOX9, INSM1*, and *RFX6*. Multiple upregulated miRNAs targeted key pancreatic TFs in *FOXA2*$^{-/-}$PPs including *NKX6.1, NEUROD1, ONECUT1, NGN3, PTF1A, GATA6, GATA4, RFX6, PDX1*, and *PAX4*. Selected miRNAs were validated using RT-qPCR.

Conclusion: Our findings indicate that FOXA2 expression during pancreatic islet development is crucial for maintaining a normal expression of key miRNAs targeting pancreatic endocrine TFs.

Accelerated epigenetic aging and DNA methylation alterations in Berardinelli-Seip Congenital Lipodystrophy

Abeer, Qannan [a*], Yosra Bejaoui [a*], Mahmoud Izadi [a], Noha A. Yousri [b,c], Aleem Razzaq [a], Arwa Aldaalis [a], George M. Martin [d], Johan Ericson [a], Jordana T. Bell [e], Steve Horvath [f,g], Junko Oshima [d,h], Andre Megarbane [i,j], Ehsan Pourkarimi [a#], Nady El Hajj [a#]

[a] College of Health and Life sciences, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.
[b] Genetic Medicine, Weill Cornell medicine-Qatar, Doha, Qatar.
[c] Computer and Systems Engineering, Alexandria University, Alexandria, Egypt.
[d] Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA 98105, USA.
[e] Department of Twin Research and Genetic Epidemiology, King's College London, London, UK.
[f] Department of Biostatistics, Fielding School of Public Health, University of California Los Angeles, Los Angeles, ca 90095, USA.
[g] Department of Human Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, ca 90095, USA.
[h] Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan.
[i] Department of Human Genetics, Gilbert and Rose-Marie chagoury School of Medicine, Lebanese American University, Byblos, Lebanon.
[j] Institut Jérôme lejeune, Paris, France.

Berardinelli–Seip Congenital Lipodystrophy type 2 (CGL2) is a very rare human genetic disorder with potential significance to the understanding of the pathobiology of aging. CGL2 patients display characteristic progeroid features and suffer from type 2 diabetes, insulin resistance, and fatty liver. In this study, we profiled genome-wide DNA methylation levels in CGL2 patients with *BSCL2* mutations to study epigenetic age acceleration and DNA methylation alterations. This analysis revealed significant age acceleration in blood DNA of CGL2 patients using various epigenetic clocks. We additionally observed an effect on the *C. elegans* lifespan following knock-down of the *BSCL2* homolog *seip-1* on a *daf-16/Foxo* mutant background. DNA methylation analysis revealed significant differentially methylated sites enriched for lyase activity, kinase regulator activity, protein kinase regulator activity, and kinase activator activity. We could also observe significant hypomethylation in the promoter of the Dual Specificity Phosphatase 22 (*DUSP22*) gene when comparing CGL2 patients vs controls. We conclude that in line with the observed progeroid features, CGL2 patients exhibit significant epigenetic age acceleration and DNA methylation alterations that might affect pathways/genes of potential relevance to the disease.

A genetically encoded BRET-based SARS-CoV-2 M^{pro} protease activity sensor

Anupriya M Geethakumari[a]*, Wesam S Ahmed[a], Saad Rasool[b], Asma Fatima[a], S.M. Nasir Uddin[a], Mustapha Aouida[a], Kabir H Biswas[a#]

[a]Division of Biological and Biomedical Sciences, College of Health & Life Sciences, Hamad Bin Khalifa University, Education City, Qatar Foundation, Doha, Qatar; [b]Division of Genomics and Precision Medicine, College of Health & Life Sciences, Hamad Bin Khalifa University, Education City, Qatar Foundation, Doha, Qatar

*Presenting author

[#]Corresponding author

The continued health, economic and social threat posed by COVID-19 has resulted in numerous efforts to develop novel therapeutic strategies to control the pandemic. Even though vaccines have been developed, the emergence of variants with a range of mutations has raised the possibility of them becoming ineffective. The SARS-CoV-2 main protease, M^{pro}, is critical for its replication and is an appealing target for designing anti-SARS-CoV-2 agents. A number of assays have been developed based on M^{pro} cleavage sequence preferences to monitor its activity. These include the usage of Fluorescence Resonance Energy Transfer (FRET)-based substrates *in vitro* and a FlipGFP reporter, one which fluoresces after M^{pro}-mediated cleavage, in live cells. Here, we have engineered a pair of genetically encoded, Bioluminescence Resonance Energy Transfer (BRET)-based sensors for detecting SARS-CoV-2 M^{pro} proteolytic activity in live cells as well as *in vitro* assays. The sensors were generated by sandwiching M^{pro} N-terminal autocleavage sites, either AVLQSGFR (short) or KTSAVLQSGFRKME (long), in between the mNeonGreen and nanoLuc proteins. Co-expression of the sensor with the M^{pro} in live cells resulted in its cleavage in a dose- and time-dependent manner while mutation of the critical C145 residue (C145A) in M^{pro} completely abrogated the sensor cleavage. Importantly, the BRET-based sensors displayed increased sensitivities and specificities as compared to the recently developed FlipGFP-based M^{pro} sensor. Additionally, the sensors recapitulated the inhibition of M^{pro} by the well-characterized pharmacological agent GC376. Further, *in vitro* assays with the BRET-based M^{pro} sensors revealed a molecular crowding-mediated increase in the rate of M^{pro} activity and a decrease in the inhibitory potential of GC376. The sensor can be utilized in studies related to drug discovery targeting the SARS-CoV-2 M^{pro} and functional genomics application to determine the effect of sequence variation in M^{pro}.

KDM5D may contribute as a critical regulator to orchestrate Y-chromosome encoding genes in acute lymphoblastic leukemia

Azin Keshavarz[a]*, Sara Chitgaran[b], Fatemeh Nasrabadi[a], Saeed Khatami[b], Vahid Moghimi[a], Reza Alemohammad[a] Ali Ghasemi [c], Moein Farshchian[a]#

[a] Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture, and Research (ACECR), Khorasan Razavi, Mashhad, Iran.

[b] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[c] Department of Pediatric Oncology-Hematology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

moein.farshchian@mail.um.ac.ir

Acute Lymphoblastic Leukemia (ALL) is one of the most widespread malignancies in the pediatric population. The KDM protein family which encodes several histone lysine demethylases plays a part in ALL, but their interactions with other genes and contributions to tumorigenesis remain unknown. In recent years, Next Generation Sequencing (NGS) technologies have provided comprehensive insight into transcriptomics. In this study, we assessed genome-wide co-expression profiling in ALL patients to reveal the hidden interplay between distinct gene clusters. The project has been carried out using public datasets available in NCBI. The accumulated number of samples is 292, consisting of 250 B-ALL patients and 42 normal samples. Transcriptome profiling was performed using the DESeq2 package. Further downstream analysis was conducted in the WGCNA (Weighted Gene Co-expression Network Analysis) package in R. Network analysis showed that *KDM5D* had the highest weight (~0.7) among all the modules constructed by WGCNA and also, and the majority of the genes associated with *KDM5D*, were located in the Y chromosome. Moreover, *KDM5D* was also highly expressed in ALL samples. Taken together, despite we could not showed a mechanistic approach for regulation of sex-related genes by this epigenetic factor but our results revealed that *KDM5D* may have a tumorigenesis role in contributing to the regulation of Y-chromosome encoding genes.

Thyroid hormone therapy in the differentiation of neural progenitor cells into cortical neurons: Potential contribution to autism spectrum disorders

Salam Salloum-Asfar[1*], Kyung Chul Shin[1], Rowaida Taha[1], Ahmed K Elsayed[1], Shahryar Khattak[2], Yongsoo Park[1], and Sara A. Abdulla[1#]

[1]Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.
[2]Royal College of Surgeons in Ireland, Bahrain

Presenting Author*, Corresponding Author[#]

Background: Thyroid hormone (T3) is crucial for brain development in utero and during the first years of life. Irregularities in T3 can affect behaviors, functioning of the nervous system and cognitive development. We observed that mothers of ASD children have thyroid irregularities and were treated for hypothyroidism throughout their pregnancy.

Objective: To investigate the role of T3 treatment in influencing neuronal development and functionality.

Methods: *In vitro* cytotoxicity of T3 was performed in neural precursor cells (NPCs), CRTD5-NPCs, by measuring cell number and growth before and after exposure to 10 different gradient concentrations of T3 (from 10nM to 1000nM). The time course consisted of 4 points: week 0 (W0), representing NPCs after 5 days of treatment, and differentiated cortical neurons measured at weeks 3 (W3), 6 (W6) and 8 (W8). To assess neuronal function, voltage-gated Ca2+ channels (VGCCs) and electrophysiology whole-cell patch clamp technologies were used. IHC staining was used to detect mature neuron markers and whole RNA-seq performed for molecular profiling of cortical neurons.

Results: VGCCs readings obtained at W3 and W6, on cells subjected to high T3 treatment, showed a lowered response to KCL. Alternatively, at W8 a higher quantity of neurons responded compared to controls, suggesting W8 neurons may have matured at a faster rate. Whole-cell patch clamp measurements at W6 exhibited increases in the percentage of neurons generating action potentials (AP). Additionally, W6 and W8 presented higher AP frequencies. W6 further showed AP peak increases and shortening of its inter-spike intervals by 50%, suggesting an increase in voltage-gated Na and K channels. Whole transcriptome showed that ion receptor pathways are upregulated in W8.

Conclusions: T3 treatments during pregnancy should be tightly regulated, as high doses can lead to irregular neuronal maturity, influencing critical systems such as the dopaminergic system, that influences learning, motor function and motivation.

Immune-phenotyping and transcriptomic profiling of peripheral blood mononuclear cells based on severity from children with autism spectrum disorders

Salam Salloum-Asfar[*], Rowaida Taha, and Sara A. Abdulla[#]

Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.

Presenting author[*], Corresponding Author [#]

Background: Transcriptomic profiling is a valuable approach to link protein and genetic information. The transcriptome apprehends a molecular genomic complexity that the DNA sequence solely does not. Some mutations alter a gene's DNA sequence but not necessarily change expression and/or protein function. Studies using peripheral blood mononuclear cells (PBMCs) from individuals with autism spectrum disorder (ASD) revealed differentially expressed transcripts compared to controls. Changes in gene expression in individuals with ASD were simultaneously differentially expressed in brain, intestinal biopsy samples, and PBMCs. This enhances detection specificity in sample types, and thus blood transcriptome-wide signatures could be used to understand ASD.

Objective: Define a transcriptomic map of ASD severity and investigate relationships between genes with enriched expression in PBMCs and ASD symptomology.

Methods: EDTA whole blood samples were collected from 38 age/gender matched children and were divided into individuals expressing more symptoms of ASD (severe) and those expressing fewer symptoms (mild). Whole transcriptome sequencing of PBMCs was performed using TruSeq total RNA Ribo-Zero Library preparation kit. Ingenuity Pathway Analysis was used to discover differentially expressed genes. To identify functional pathways influenced by the combinatorial effects of the long noncoding RNAs analysis, LncPath package in R was used.

Results: An average of 97.75% of reads from each sample was uniquely mapped. From 47,314 genes, 19,939 protein-coding genes and 27,375 non-coding genes were identified in children with ASD and healthy controls. We defined genes expressed in PBMCs from either severe/mild ASD children and healthy controls. Our transcriptomic signature discovered novel genes and molecular programs that control the regulation of cytokine production and neuroinflammation. Functional enrichment analysis demonstrated that differential expressed genes were significantly enriched in inflammation/immune response, mitochondrion-related function and oxidative phosphorylation.

Conclusion: Transcriptomic profiling provides an insight to the etiology of ASD neuroinflammation and the possibilities of developing interventions based on severity.