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MECHANISTIC AND EXPLORATORY RESEARCH

The association between vitamin D levels in pregnancy and vitamin D receptor polymorphisms in offspring with autism spectrum disorder

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Background: Studies have correlated gestational vitamin D (VD) status and child autism spectrum disorder (ASD). However, the influence of offspring vitamin D receptor (VDR) genotype polymorphisms has not been studied. Therefore, we aimed to investigate the association between gestational VD and offspring VDR polymorphisms in ASD. Method: As part of the Vitamin D in Pregnancy study, maternal serum samples were collected before 16 weeks (early) and at 28-32 (late) weeks gestation, where VD concentration was analysed via radioimmunoassay. Offspring VDR genotype (Bism1,Fok1,Apa1,Taq1) was determined from neonate bloodspots. At 11 years, offspring were assessed for ASD symptomatology using the Australian Scale for Autism Spectrum Conditions (ASASC). Results: 192 mother-child pairs were included. Gestational VD levels in early and late pregnancy were not associated with total ASASC scores (all p>0.05). No association was found with ASASC score and any VDR genotype (all p>0.05). In secondary analyses of specific subscales (understanding emotion,fact-orienting,sensory sensitivity,social communication,rigid adherence to routine), higher VD was associated with decreased fact-orienting scores in males (β:-0.05,95%CI-0.08,-0.01,p=0.02). No other ASASC subscale was affected in either sex (all p>0.05). The Taq1 VDR polymorphism was linked to the fact-orienting subscale, whereby the homozygous recessive genotype was associated with a 2.5 unit score increase over the homozygous dominant genotype (β:2.52,95%CI0.36,4.69,p=0.02). No interaction was observed with gestational VD. Conclusions: There was no association between gestational VD levels, VDR polymorphisms, and total ASASC scores. However, gestational VD and the Taq1 VDR genotype may influence specific ASD traits, highlighting potential genetic links to behavioural changes in ASD.

Placental transcriptional signature of impaired mitochondrial function and autism spectrum disorder in the Barwon Infant Study

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Autism Spectrum Disorder (ASD) is a serious neurodevelopmental condition characterised by impaired communication and repetitive behaviour. The aetiology of ASD is complex, likely involving multiple genes and interactions with environmental factors. Growing evidence implicates impaired mitochondrial function as one of the key mechanisms underlying the pathophysiology of ASD. We conducted a nested case-cohort study to investigate the placental transcriptional signature and pathways among infants subsequently diagnosed with ASD against the DSM-5 criteria (n=43) in comparison to a random sub-cohort (n=120) in the Barwon Infant Study. RNA sequencing was performed using pooled, multiple biopsies of each placenta. A total of 1,644 differentially expressed genes (DEGs; FDR<0.05) were identified in placenta from children diagnosed with ASD compared to the random subcohort. These genes contributed to the enrichment of Canonical Pathways, Gene Ontology enriched terms, KEGG and REACTOME pathways related to mitochondrial function, oxidative stress and ribosomal pathways. Impaired mitochondrial function is a known feature of ASD pathophysiology in older children and adults, but the causes and age of onset of this impaired function are unclear6 The placenta is a key determinant and regulator of fetal brain development. Thus, a placental transcriptome signature indicative of both xenobiotic susceptibility and impaired mitochondrial function among infants subsequently diagnosed ASD has profound impact on public health and mechanistic research targeting the prenatal window for autism prevention.

Single-cell sequencing clarifies the landscape of regulatory mechanisms following influenza infection of Adamts7 knock-out (KO) mice.

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Background 'A disintegrin and metalloproteinase with thrombospondin motifs 7' (ADAMTS7) is a large and secreted extracellular matrix enzyme. Current findings indicate that changes in expression may be associated with susceptibility to coronary artery disease and arthritis. Moreover the in vitro silencing of ADAMTS7 impacts influenza virus replication via the NF-κB pathway. Little known about the role of ADAMTS7 in influenza virus immunopathogenesis. Methods Splenocytes from wild-type (WT) and Adamts7 knock-out (KO) were harvested from day 3 infected mice. Subsequently, single cell cDNA libraries from WT and KO samples were established using the BD Rhapsody™ Whole Transcriptome Analysis (WTA) system. Both cDNA libraries were sequenced on the Illumina NovaSeq6000 . Downstream single cell processing, GO ontology and trajectory analyses were performed using the Seurat, Clusterprolifer and Monocle3 packages, respectively. Results A total of 16 cell subsets were identified in both Adamts7 KO and WT mice. KO mice showed an increase in the plasmacytoid dendritic cells (pDC) subset when compared with WT controls. The GO ontology and gene set enrichment analysis (GSEA) suggested that changes in pDCs may reflect perturbations in neutrophil migration and chemotaxis. Subclustering for neutrophils revealed 3 different subsets that require further investigation (activated neutrophils, conventional neutrophils and CCR3+ neutrophils). Conclusion Our results suggest that the lack of ADAMTS7 expression may regulate the innate immune response against influenza infection. Further investigation for individual cell subset required to understand the underlying mechanism of action.

Identification of Natural Killer Cells in Zebrafish

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Zebrafish is an invaluable model for studying blood and immune cell development and function, with strong conservation of cells and key genes with humans. However, the natural killer (NK) cell population has been understudied in zebrafish. This study seeks to address this knowledge gap by developing markers to investigate NK cell biology in zebrafish. RNA probes for several candidate genes were developed to study their expression during early zebrafish embryogenesis, particularly in mutants with altered NK cell production. The NK lysin genes, nkl2 and nkl4, and the transcription factor zbtb32 were found to be specifically expressed in the thymus from 5 dpf. The expression of another transcription factor, tbet, was first seen in the forebrain at 3 dpf and then in thymus from 5 dpf. Finally, the non-specific cytotoxic receptor gene (nccrp-1) was found to be expressed in the gut from 3 dpf, shifting to the thymus at 7 dpf. A significant increase in expression was observed in all these genes except for nccrp-1 in zebrafish harbouring hyperactive Jak3 mutation in which an increase production of NK cells was expected. The study has identified potential markers for identifying NK cells in zebrafish. Amongst these nkl2, nkl4 and zbtb32 exhibit expression making them good candidates for the future generation of transgenic lines in which NK cell populations are fluorescently tagged. This would enhance understanding of fish NK cells and establish zebrafish as a relevant model for this aspect of immunology and paving the way for new insights and potential therapeutic innovations.

Designing gradient bioinks for the musculoskeletal interface

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A significant characteristic of the musculoskeletal system is the contiguous interface where the soft connective tissues such as cartilage, tendon and ligaments connect to the crystalline bone. This transition is a unique zone that ensures the stability of the joints by creating support around the joints. These soft-to-hard tissue interfaces are prone to injury and are limited by their ability to self-repair. Thus, increasing the chances of risk to damage and decreasing the functionality. Restoring such gradient interfaces remains a major challenge in interfacial tissue engineering because of their complex and dynamic nature. A novel hybrid hyaluronic acid and peptide-based hydrogel approach were taken to construct a biomimetic tissue scaffold of defined porosity, with growth factors incorporated to functionalise the material, we then used extrusion-based 3D bioprinting technique to fabricate the physical gradients. In this method, multipotent stem cells were embedded into the hydrogel, and a controlled biochemical stimulus promoted cell differentiation. The mechanical properties of the tissue scaffold are optimised to match the natural tissue to withstand forces until the remodelling process ends as the joints are exposed to different mechanical stresses regularly. The properties of the hydrogels were tested using Differential scanning calorimetry, Rheology, Fourier-transform-infrared-microscopy, Cryo-SEM and Atomic-Force-Microscopy. We demonstrate that the synthesized novel bio-ink can bio-print tissue constructs with both physical and chemical gradients. This research has recognised a potential novel bio-ink that can fabricate gradient tissue constructs that mimic the host tissue, supporting cell attachment and cell proliferation to promote musculoskeletal interface repair.

Transmission decline of Plasmodium falciparum is associated with genome-wide reduction in diversity in a hyperendemic area of Papua New Guinea

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Malaria genomic surveillance is crucial to understand how control strategies impact parasite populations and to detect novel variants. In Papua New Guinea there have been extensive control measures since 2006, which initially resulted in a substantial decline in parasite prevalence. More recently however, the emergence of artemisinin resistant parasites and rebounds in parasite prevalence have been observed. Relatedness analyses of SNP barcodes revealed the presence of several clonal lineages after transmission reduced. Control initially fragmented the parasite population, however rebound enabled the expansion and spread of clonal lineages. We aimed to determine whether resurgence was the result of adaptation of the parasite population to control efforts. Thus we conducted whole genome sequencing of 110 P. falciparum isolates from the same time points in East Sepik and Madang Province's to explore genome-wide diversity, population structure and patterns of selection over time to evaluate whether gene adaptations may be driving the expansion of certain lineages observed in the SNP barcode analysis. We used an in-house bioinformatic pipeline to identify high-quality genotypes across the genome. We analysed diversity using various metrics including Fst, phylogenetics, Tajima's D and explored the population structure using Discriminate Analysis for Principal Components. Pairwise Identity-by-Decent was explored using IsoRelate to observe genome-wide signatures of diversity and selection. Lastly, we looked at demographic changes over time using BEAST. This study highlights the utility of whole genome sequence data to explore the changing population structure and possible evolutionary mechanisms parasite populations use to overcome control efforts, as countries move towards elimination.

Investigating the role of SNARE proteins in Plasmodium falciparum rhoptry exocytosis

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Malaria, a devastating infectious disease caused by Plasmodium parasites, poses a significant global health challenge due to the rise of drug-resistant strains. This project aims to tackle this issue by exploring a potential new avenue for drug development. The ability of the parasite to invade and replicate within red blood cells (RBCs) is critical for its survival and the progression of the disease. This complex process involves the secretion of various proteins from a specialized organelle called the rhoptry into the RBC. However, the mechanisms governing rhoptry fusion with the parasite membrane to facilitate secretion are not fully understood. SNARE proteins, known as tethering proteins, assist in the docking and fusion of vesicles in eukaryotes to facilitate content release. It is hypothesized that SNARE proteins are also involved in rhoptry organelle fusion and secretion. This study investigates the role of SNARE proteins in rhoptry secretion by using reverse genetics to modify the expression of the SNARE gene. Specifically, gene targeting constructs for engineering the SNARE locus have been developed and are currently being transfected into Plasmodium parasites. The integration of these constructs into the correct locus will be confirmed, and the expression of the SNARE gene can be regulated to analyse its consequences on invasion and rhoptry secretion. If SNARE proteins are crucial for rhoptry function, targeting them could hinder RBC invasion, potentially reducing parasite propagation. This study could unveil SNARE proteins as a novel drug target against Plasmodium falciparum malaria, offering insights into parasite biology and drug development.

Characterizing the role of kat5 in zebrafish development and disease

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Over 80% of skin cancer fatalities are caused by melanoma. New targeted therapies and immunotherapies have significantly improved clinical outcomes in the last decade, but most patients ultimately succumb to their disease because of primary or acquired resistance. In order to find more effective approaches to treating melanoma, it is imperative to better understand the molecular drivers of progression and therapeutic response. Mutations are prevalent in melanoma cells because of extensive DNA damage caused by ultraviolet (UV) light. These mutations affect two main classes of disease drivers - oncogenes that activate signal transduction pathways promoting cell proliferation or preventing cell death, and tumour suppressor genes that activate pathways inducing cell cycle arrest, senescence, or cell death. However, there is now strong evidence that the evolution, progression rates, and treatment responses of melanomas are also associated with additional genetic events (eg. mutations, chromosomal loss/gains) impacting a class of genes termed disease modifiers, many of which function as epigenetic regulators of gene expression. One such modifier is the histone variant H2A.Z, aberrant incorporation of histone variants into nucleosomes was recently shown to contribute to melanoma development and progression. Replacement of H2A in nucleosomes with H2A.Z is mediated by one of two highly conserved protein complexes, TIP60 and SRCAP, which share many subunits. Based on publicly available data on the mutational landscape of human melanomas, our laboratory recently identified 2 common subunits of these complexes, VPS72 and YEATS4, as recurrently amplified and/or overexpressed with 22% and 9% of cases respectively.