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Treatments for those who have none platform: from Western Australia to nationwide

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Background: Despite individual low prevalence, rare diseases (>7,000) collectively affect a substantial population, estimated to be between 263 million and 446 million worldwide. Most (95%) rare diseases do not have approved treatment and approximately 45% cause neurological disorders. We initiated the “Treatment for those who have none” platform for Western Australian children with a rare disease in 2021, with support from a Channel 7 Telethon Trust research grant. The purpose of the platform is to accelerate treatments from bench to bedside for rare diseases using antisense oligomer-mediated modulation of gene expression.

Methods: Through the Rare Care Centre, Perth Children Hospital, we recruited patients diagnosed with a rare disease, and analysed the consequences of the mutation at the RNA levels. Based on these observations and known pathogenic mechanisms, we selected three patients, two with neurological disorders and one with kidney disease, for ASO design and assessment. We designed ASOs to either increase levels of gene expression for the children with neurological problems or redirect pre-mRNA splicing to induce a truncated protein with some functionality for child with kidney disease.

Results: Within 18 months, we have designed ASOs and performed proof-of-concept studies in patient-derived cells for three rare diseases Kleefstra syndrome, Birk-Landau-Perez Syndrome and Alport syndrome.

Conclusion: With this experience, we are now extending this platform nationwide through collaborations with Royal Brisbane Hospital, Queensland University Technology, University of New South Wales, Garvan Institute, Sydney Children Hospital network, Murdoch Children Research Institute, and Royal Hobert Hospital. We aim to integrate this program into the healthcare system as a treatment route for rare diseases.

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Testing gene replacement therapy in retinitis pigmentosa and Leber congenital amaurosis

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Background: Retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) are some of the most common forms of inherited retinal dystrophy (IRD) leading to photoreceptor dysfunction and vision loss. Autosomal recessive forms potentially suitable for gene replacement therapy as used for treatment of RPE65 IRD, are caused by variants in genes including RPGR, AIPL1, RLBP1, PDE6B and RPGRIP1. Due to high clinical and genetic heterogeneity, there is a need to ascertain relevant biomarkers to assist genetic variant classification and provide patients with a diagnosis and opportunities for therapy. In this study, we aim to establish human models for elucidating disease mechanisms and testing novel AAV gene replacement therapies for variants in suitable RP and LCA causing-genes using induced pluripotent stem cells (iPSCs) differentiated to retinal organoids.
Methods: We generated patient-derived and CRISPR/Cas9 engineered iPSC lines carrying different variants, including pathogenic and variants of uncertain significance (VUS), in two genes modelling RP and LCA, respectively. All iPSC lines were differentiated to 32-week-old retinal organoids for immunohistochemistry, western blotting and transcriptomic analysis. For therapy testing, 21-week-old variant retinal organoids were transduced with AAV carrying wild-type cDNA of the retinal gene.
Results: Compared with controls, all variant organoids had diminished/reduced expression along with abnormal photoreceptor cell staining. Furthermore, these photoreceptors displayed inadequate function, either due to mislocalisation, aberrant binding characteristics or accumulation of enzyme substrate due to inactivity. At the transcript level, gene set enrichment analysis showed enrichment of abnormal transcriptomic profiles in all forms of mutant organoids compared with controls. Two months post AAV therapy of variant organoids, we observed induction of transgene protein expression and improved photoreceptor morphology compared to equivalent organoids that did not receive therapy.
Conclusion: This study demonstrates utility of iPSC-retinal organoids for functional genomics and therapy testing in retinitis pigmentosa and Leber congenital amaurosis.

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Pre-natal AAV gene therapy for HBSL disease in Dars1 transgenic mice.

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Background: Gene therapy is a promising treatment for Hypomyelination with Brainstem and Spinal cord involvement and Leg Spasticity (HBSL), a potentially fatal disease caused by DARS1 gene mutations impacting brain and spinal cord white matter. We developed gene augmentation vectors (AAV.PHP.eB-hDARS1) for targeted CNS expression of human DARS1. In our existing set of transgenic mouse models, these vectors improved HBSL symptoms when delivered systemically. However, earlier intervention is needed to achieve a complete rescue. Here, we established a novel HBSL mouse model ('Massi') carrying two HBSL patient variants (Dars1A274V/D367Y), which suffers pre-natal mortality, to explore the potential of in-utero gene therapy. For minimal-invasive gene delivery, we tested the safety and efficacy of ultrasound-guided pre-natal AAV administration.
Methods: Dars1A274V/D367Y Massi mice were characterised using molecular, behavioural, histological and biochemical measurements. Using AAV.PHP.eB-eGFP, we assessed the safety and CNS-targeting of UltraSound-guided, PerCutaneous, Foetal IntraPeritoneal (USPCFIP) injections at gestation day 14.
Results: Dars1A274V/D367Y Massi mice show mild to moderate adult symptoms but high pre-natal mortality with a lower live birth rate (~20%) than expected from the Mendelian pattern of inheritance (50%). Preliminary results exploring USPCFIP delivery of AAV.PHP.eB-eGFP led to a 75% postnatal death rate in the eGFP-treated group, compared to 25% in controls. Vector copies were found in 2/17 newborn brains, while eGFP transgene expression was observed in 3/6 livers but not in brains.
Conclusion: Massi (Dars1A274V/D367Y) mice model moderate HBSL disease but display high pre-natal mortality, making them a valuable tool to study AAV-mediated in-utero gene delivery. USPCFIP injection allows CNS transduction in foetuses, but challenges include target accuracy and potential eGFP toxicity. Furthermore, rapid cell division of the developing CNS, which might obscure transgene expression and detection, is an important consideration for the future development of appropriate in-utero gene therapy strategies.

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Gene Therapy for Brittle Bone Disease

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Background: Brittle bone disease (also known as osteogenesis imperfecta or OI) is a congenital bone fragility condition that leads to frequent fractures. Current pharmacotherapies do not address the underlying genetic cause and we have conceptualised gene repair strategies to correct disease-causing mutations. As a proof-of-concept, we have developed a novel approach for treating a patient with a severe OI caused by a 20 bp deletion (Δ20) in COL1A1.
Methods: To model the disease, a human cell line was created possessing the COL1A1Δ20 mutation that produces a frameshift and significant C-terminal missense readthrough. In addition, we generated a Col1a1Δ20/+ mutant mouse with an analogous mutation. One challenge with CRISPR/Cas9 gene editing is efficiency, however we identified that this poison protein allele could be susceptible to gene disruption; a targeted SaCas9 gene disruption (non-homologous end joining) approach was trialled. Preclinical testing in the Col1a1Δ20/+ mouse will be performed using a single-vector AAV approach, making use of our prior research targeting gene constructs to the bone.
Results: MicroCT analysis of bones isolated from the Col1a1Δ20/+ mouse demonstrated reduced bone mass in the axial and appendicular skeleton. 4-point-bending and compression testing showed reduced biomechanical strength in the tibiae and vertebrae. SaCas9 targeting of the COL1A1Δ20 mutant showed >70% disruption of the problematic readthrough allele with 0% effect in the wild type COL1A1 locus. Data from cell tracking studies using fluorescent reporter mice showed high efficiency, selectivity, and persistence in the bone compartment with an AAV8 approach that will be used for subsequent rescue studies.
Conclusion: We have generated novel cell and mouse models of a patient OI mutation. Our gene therapy approach has the potential to be transformative for OI patients and could also be adapted to treat to other conditions caused by dominant negative gene products.

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PeptAIde: Accelerating Antimicrobial Peptide Discovery Using AI Ensemble Techniques

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Background: The rapid rise of antimicrobial resistance (AMR) poses a critical global health challenge, with projections indicating up to 10 million deaths annually by 2050 if current trends continue. Antimicrobial peptides (AMPs) offer a promising alternative to conventional antibiotics, with broad-spectrum activity, rapid action, and reduced resistance development. However, discovering natural AMPs is a complex, time-consuming, and costly process.
Methods: This study leveraged pattern recognition (PR) and artificial intelligence (AI) to discover novel encrypted AMPs within phage biological information. We built a PR and AI ensemble approach, integrating various machine learning techniques to improve prediction accuracy. Our PR algorithm, inspired by Ma et al. (2022) and Melo et al. (2021), detects AMP-like sequences based on physicochemical properties such as charge and hydrophobicity. Deep-learning models were then applied to identify sequences with low similarity to known AMPs and predict activity based on the amino acid sequence context.
We analysed the entire phage and viral pan-proteome, comprising 1.5 million proteins from UniProt. Peptides were grouped based on sequence similarity, charge, and hydrophobicity, selecting the top predictions for in vitro testing.
Results: Our ensemble approach identified hundreds of millions of peptides, narrowing down to 23 high-scoring AMPs for empirical testing. Initial screenings confirmed the antimicrobial activity of this subset, significantly reducing survival rates of multi-drug or pan-resistant pathogens like Pseudomonas aeruginosa and Staphylococcus aureus. These peptides demonstrated broad-spectrum activity and lower toxicity toward mammalian cells, and based on our preliminary results, are among the most potent AMPs reported to date. making them viable candidates for further pre-clinical development.
Conclusion: Our AI ensemble approach effectively identified novel AMPs within phage proteomes, showcasing potential as next-generation antimicrobials. These findings support the feasibility of using advanced computational tools to accelerate AMP discovery, providing a valuable framework for the inexpensive development of bioinspired antimicrobials to combat AMR.

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Modelling PROM1 retinal disease in a mouse model and human retinal organoids

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Introduction: Disease-causing variants in PROM1 lead to inherited retinal dystrophies (IRD), characterized by both cone and rod dysfunction with various retinal sub-phenotypes reported. The aim of this study was to investigate disease mechanism in mouse and patient-derived retinal organoid model systems and to aid pre-clinical assessment of novel therapy approaches.
Methods: To study PROM1 disease, we used two models: a mouse model and patient-derived induced pluripotent stem cells (iPSCs) differentiated into retinal organoids (ROs). Serial electroretinograms (ERG) and optical coherence tomography (OCT) were conducted from P21 to P180 and compared to wild-type mice. Histological analyses of mouse retinal sections were performed at postnatal days (P) 14 to P90.
Immunohistochemistry (IHC) assays targeted cone and rod-specific markers, as well as glial markers. Retinal organoids matured until day 210 (D210) were collected for morphological assessment, IHC and RNA extraction. Transcriptomics was performed using RNA-Seq to investigate differentially expressed genes (DEGs).
Results: Prom1 mutant mice showed retinal degeneration starting from P14 over a 6-month period compared to controls, based on OCT, ERG, histological, immunohistochemical studies. OCT quantification showed a significant difference in retinal thickness at P30, and by P180, the mean retinal thickness difference was 65% of wild type. Electrophysiology indicated cone dysfunction at P21, shown by reduced flicker function amplitude. Histology revealed reduced outer nuclear cell density at P14. ROs with pathogenic bi-allelic PROM1 variants exhibited an absence of huPROM1 and a reduction in cone numbers compared to controls. Transcriptomics revealed significant downregulation in pathways related to angiogenesis, eye morphogenesis, and bleb assembly.
Conclusion: Autosomal recessive PROM1 IRD causes both rod and cone dysfunction, as demonstrated in a mouse model and patient-derived ROs. Evidence from this study suggests that cone dysfunction may precede rod loss, providing insights into the varied phenotypic and genotypic presentations of the disease and biomarkers for therapy assessment.

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Using behavioural theories to implement novel communication resources for healthcare professionals

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Background: Healthcare professionals (HCPs) are responsible for communicating with families of children with hard-to-treat cancers on issues surrounding experimental treatments and paediatric precision medicine. Co-designing with HCPs enables the simultaneous development of targeted strategies and optimal uptake of these resources. The Theoretical Domains Framework (TDF) is an innovative implementation science framework that facilitates understanding of the underlying determinants of behaviour, such that we can find the best approaches to facilitate change. We used the TDF to identify determinants of behaviour relevant to HCP communication skills in complex paediatric cancer treatment, and to develop accessible strategies to address implementation problems and support resource dissemination.
Methods: We interviewed Australian HCPs who had direct responsibilities in managing children/adolescents with a hard-to-treat cancer within the past 24 months. Interview transcripts were qualitatively coded to domains of the TDF, and behaviour change techniques were identified. Based on these interviews, we developed a video resource for HCPs as well as recognised strategies to achieve optimal resource dissemination.
Results: We interviewed 10 oncologists, seven nurses, and three social workers who identified several challenges for communication with families including: balancing information provision while maintaining realistic hope; managing their own uncertainty; and nurses and allied health workers being under-utilised during conversations with families. Key determinants of behaviour were environmental context and resources, knowledge, and skills. Behaviour change techniques included seeking support from colleagues, role-playing difficult conversations, and understanding the potential impact these discussions have on families.
Conclusion: Resources are needed to provide inclusive support for all types of HCPs in communication with families of children with hard-to-treat cancers, particularly when using precision medicine in paediatric cancer. By identifying implementation strategies, our research may innovate the integration of future resources and make them accessible in HCP training, such that best-practice person-centred care is achieved with a wider range of families.

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Adapting SIBS-ONLINE for Australian siblings of patients with genetic epilepsy

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Background: Siblings of children with chronic illness can experience profound mental health difficulties and poor family functioning. Psychological support for siblings is currently lacking in Australia. SIBS is a group, cognitive behavioural therapy intervention developed in Norway which has been shown to improve sibling mental health and sibling-parent communication. We conducted a series of studies to inform the adaptation of SIBS for Australian families of children with chronic illness.
Methods: Forty-five siblings (M age=15.4 years, SD=3.3 years; 60% female) completed questionnaires which included visual analogue scales of wellbeing. Ten (22.7%) siblings completed an interview and 86 parents completed qualitative questionnaires to determine perceived need for, and central components of, psychological support. We analyzed this data with thematic analysis.
Results: Over one-third (n=17, 43%) of siblings reported clinically-relevant anxiety and 25% (n=10) reported clinically-relevant distress. In the interviews siblings revealed that they often undertook caring responsibilities for their brother or sister, and that these could be "taxing" with some siblings expressing anxiety about being a "fulltime carer" in later-life. Siblings and parents qualitatively reported that targeted mental health interventions were "desperately needed". Siblings emphasised that they wanted to connect with other Australian siblings so they could "feel less alone", be able to communicate with their parents about their challenges and emotions, and have support delivered online from the "safety" of their bedroom.
Conclusion: Our findings show that family-based psychosocial interventions which target the sibling-parent relationship, such as SIBS, are strongly desired by siblings and parents alike. Adaptations to SIBS for Australian families involve delivering the intervention via videoconferencing (SIBS-ONLINE) and including a greater emphasis on siblings' caring responsibilities and uncertainties for the future. We are now piloting SIBS-ONLINE for Australian families, beginning with siblings and parents of

patients with genetic epilepsy given the extremely high mental health concerns among these families.

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Partnering with consumers to advance research and impact for inherited retinal diseases

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Background: Engaging with the community strengthens the research processes. Yet, this doesn't often occur when determining what research should be prioritised. We sought to identify the research priorities for inherited retinal diseases (IRDs) – a currently untreatable group of genetic eye conditions that are commonly diagnosed in childhood - from the perspective of key stakeholders (i.e., individuals who have an IRD, caregivers, and health professionals).
Methods: We conducted a Priority Setting Partnership (PSP) following the James Lind Alliance methodology. To guide our PSP, we formed a multidisciplinary and lived experience steering group. Via survey, stakeholders submitted questions they had about IRDs. We consolidated submissions into overarching questions and confirmed whether they remained unanswered by performing a literature review. In a second survey, stakeholders voted for the unanswered questions they deemed most important. In August, we will hold two online workshops where stakeholders will discuss the highest ranked questions and finalise the top 10 research priorities for IRDs in Australia.
Results: We closed our first survey with 42 overarching questions from 227 submissions. Only one question was deemed answered in the literature. From the 151 participants (lived experience=89%) who voted in the second survey, the highest ranked questions related to treatment to: prevent, slow or stop vision loss (#1 priority) or restore vision (#2). Other highly ranked questions were around anticipated progression of vision loss (#3), the psychological impact of having an IRD and effective supports for patients (#4) and carers (#6), and access to genetic testing (#5).
Conclusion: In our presentation, we will share the final top 10 research priorities, alongside our learnings from partnering with individuals with lived experience. We will use the identified priorities to advocate for research that aligns with what the community consider most important. This will maximise research impact and utilisation of health dollars.

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Trio germline sequencing in childhood cancer: Healthcare professionals' perspectives from PREDICT

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Background: Germline genomic sequencing (GS) is increasingly offered to children with cancer. To optimize integration in routine care, ongoing assessment of barriers to implementation and better understanding of the perspectives and experiences of healthcare professionals delivering this testing is needed.
Methods: Healthcare professionals delivering germline GS to newly-diagnosed paediatric and adolescent patients with cancer via the PREDICT study were invited to complete questionnaires including qualitative and quantitative items. A healthcare professional at each site recorded reasons for eligible families' non-enrolment in PREDICT to interrogate barriers to recruitment. Quantitative data were analysed via descriptive statistics. Qualitative data were analysed via inductive content analysis. Results were then integrated for interpretation.
Results: Thirty-three healthcare professionals participated (33/70 invited, 47% participation rate) including 23 oncology professionals and 10 genetics professionals. Healthcare professionals perceived taking part in PREDICT was beneficial to participating and future families, and that both perceptions of personal benefit and altruism were drivers of family uptake. However, they also described concerns about workforce capacity and the potential for results to cause distress for families given the trio design and the high-stress diagnosis setting. Barriers to recruitment included clinician reluctance, family reluctance, and logistical challenges. Most healthcare professionals rated their knowledge of genetics/genomics as 'good,' yet in relation to germline results, few were 'very confident' in: interpreting (29%), explaining (32%), making treatment recommendations (9.7%), and

providing psychosocial support to families (29%). Healthcare professionals recognized a need for further training in these areas for trainees, yet fewer were interested in receiving further education/training for themselves.
Conclusion: Successful implementation of routine germline GS for children with cancer will require targeted strategies to address logistical issues and alleviate potential negative psychosocial impacts for families. Recognizing escalating demand on genetics experts, upskilling of the current workforce and involvement of a broader spectrum of healthcare professionals is warranted.

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The consumer group landscape: identifying advocacy gaps and improving precision medicine outcomes

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Consumers are essential to ensuring research translation is meaningful. As paediatric precision medicine (PPM) advances, there is a need to integrate psychosocial care to address the unique challenges faced by families, therefore emphasising the necessity for consumer engagement. Current efforts to engage consumer groups are often fragmented, creating silos that hinder comprehensive advocacy and support. This causes difficulty for researchers to practice effective consumer engagement and burdens consumers.
This study aims to explore the landscape of consumer groups across Sydney Children's Hospital Network (SCHN) related to PPM, focusing on understanding their dynamics, functions, and capacity for collaboration. By identifying these elements, we seek to uncover opportunities to reduce silos and improve psychosocial integration within the precision medicine framework.
Phase one of this study involved conducting an Environmental Scan (ES) to identify consumer groups and organisations throughout the SCHN relevant to PPM. An online search was conducted and supplemented with groups shared by PPM clinicians and researchers. To gain further insights into group dynamics, characteristics, and collaborative potential, interviews and surveys will be conducted in Phase two.
The ES identified forty-one consumer groups and organisations. The primary areas advocated for included epilepsy, cancer, cerebral palsy and general mixed age chronic illness and disability. The role and purpose of each group was identified for most of the groups. However, we were unable to determine information about group characteristics and collaborative capacity.
This study has been able to identify gaps in advocacy, such as psychosocial care surrounding PPM and resulted in a comprehensive map of the current consumer landscape. This foundational knowledge is essential, however, Phase two will reveal actionable knowledge about how to champion and collaborate with these groups. This may address the current fractures in consumer engagement, with the aim of also reducing duplication, inefficiency and the overburdening of consumers.

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Paediatric service navigation to address life challenges: a mixed-methods study

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Background: Healthcare providers face significant barriers identifying and responding to adversity for children, young people, and their families. Innovative solutions to improve early identification of and intervention for adversity are required. This study aimed to assess feasibility and acceptability of a service navigation program for families experiencing adversity.

Methods: We conducted a mixed-methods implementation and evaluation study including: 1) co-design of an integrated health and social model of care; 2) implementation of a service navigator program in a community health centre; and 3) mixed-methods program evaluation. Participants included caregivers with children aged 0-18 years referred to service navigation, medical, allied health, legal and social service providers. Co-design workshops were conducted with relevant stakeholders to identify research priorities and develop a model of care including service navigation. The program was implemented during 2022-2023 in Marrickville, Sydney. Quantitative data on participant and program characteristics were collected and analysed and semi-structured interviews were undertaken to explore experiences of service navigation.

Results: Most caregivers were born in Australia (27/33, 82%), with (12/33, 36%) identifying as Aboriginal and (5/33,15%) speaking languages other than English at home. Common needs included financial (25/33, 64%), housing and mental health/substance use, both (19/33, 49%). Participants found the program acceptable due to trust in the service navigator. They reported efficient and streamlined referrals processes. Barriers to utilising the program included complex care needs requiring intensive navigation, high staff turnover, and poor confidence in sustainability of the navigation service.

Conclusion: A service navigation program for families experiencing adversity was feasible and acceptable, however integrated models of care and services should accommodate a spectrum of complex care needs with fluctuating workforce capacity. Approaches to address this include exploring other early identification and intervention strategies for family adversity such as use of digital platforms for integrated care and service navigation.

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Harnessing liquid biopsy to unveil somatic RAS-MEK pathway variants in extracranial AVMs

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Background: Arterio-venous malformations (AVMs) are rare congenital high-flow vascular anomalies characterized by abnormal direct artery-to-vein communications, lacking intervening capillaries. Complex extracranial AVMs, typically have a limited response to standard treatments such as sclerotherapy or surgery, leading to a progressive disabling pathology with lifelong consequences that include chronic pain, deformity, and disfunction. Somatic variants in the RAS-MEK pathway are implicated in AVMs, generating interest in the role of MEK inhibitors to treat this condition. However, open biopsy for molecular characterisation carries a potentially life-threatening bleeding risk.
We aimed to evaluate the effectiveness of liquid biopsy for somatic genotyping of AVMs in children and young adults. We hypotheses that liquid biopsy from efferent draining veins is a reproducible and feasible technique for obtaining DNA from AVM, and that detection of the underlying somatic mosaic variants from AVMs can assist in directing targeted pharmacotherapy.
Methods: 10 patients with complex AVMs for which endovascular treatment or diagnostic catheter angiography was being undertaken were enrolled and 15 blood samples collected (11 efferent vein, 3 synchronous peripheral vein, and 1 peripheral vein only). Ultra-deep panel next-generation sequencing was conducted on cell-free DNA (cfDNA), followed by error-correction sequencing data analysis and variant calling.
Results: Pathogenic variants were identified in 12 samples from 8 patients (levels 0.11%-20.06%). No procedural complications occurred from efferent sampling. Three patients with identified variants began genotype-directed pharmacotherapy, demonstrating marked clinical improvement.
Variant levels inversely correlated with the sampling distance from the AVM nidus (p=0.004). Similarly, variant levels in synchronous peripheral collections were reduced by 33-100% in 3 patients (p=0.035).
Conclusion: Somatic variants of the RAS-MEK pathway are common in extracranial AVMs and can be readily identified using efferent vein liquid biopsy, albeit with a moderate yield in peripheral samples. This approach reduces bleeding risk, enabling broader access to targeted pharmacotherapy for AVM patients.


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Cryptico: A Computational Approach for Exploring Cryptons in Search of Novel Therapeutics

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Background: The development of new cancer therapies is constrained by the proteome's limited druggability, estimated at only 10-15%. Additionally, the increasing recognition of non-coding RNAs' roles in disease underscores the urgency for innovative therapeutics that move beyond conventional protein-targeting strategies. The emergence of RNA-targeting small molecules presents a promising avenue for novel therapeutic approaches, potentially expanding the range of actionable targets. Recent advancements include the use of splicing modulator compounds (SMCs) to suppress gene expression by introducing deleterious cryptic exons (cryptons) in a sequence specific manner.
Methods: In this study, we developed a computational method to systematically identify potential targets for SMCs, specifically cryptons within the human genome. This method combines deep learning with high-throughput RNA-sequencing data from human tissues to discover high confidence putative cryptons.
Results: We detected around 50k cryptons and predicted their therapeutic potential based on their ability to disrupt gene expression through PTCs, frameshift mutations, or the alterations of protein folding due to the introduction of novel peptide chains. Furthermore, our analysis revealed 41,664 previously unannotated cryptons, some of which are predicted to interfere with several oncogenes traditionally considered undruggable. We further validated the existence of several of cryptons in therapeutically relevant genes, by engineering one of the spliceosome components to match a specific cryptons 3' splice sites.
Conclusion: Our findings provide a valuable resource for exploring potential therapeutic targets of SMCs and lay the groundwork for future drug screening efforts.

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Inferring the life history of NTRK-fusion paediatric tumours

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Background: Many paediatric cancer tumours are driven by rearrangements in neurotrophic tyrosine receptor kinase (NTRK) genes (NTRK1, NTRK2 and NTRK3). While several inhibitors targeting tropomyosin receptor kinases family genes have been either approved or are at an advanced stage of clinical development, many paediatric cancer patients develop resistance to these inhibitors. Tumour evolution studies in adult cancers suggest polyclonal tumours likely to develop therapy resistance. We hypothesized that characterizing the tumour evolution of pediatric cancers with NTRK fusions might reveal insights into resistance mechanisms.
Methods: In our extended ZERO-Childhood Cancer cohort, we identified 26 tumours (from 24 children) with NTRK-fusion events. This cohort includes central nervous system tumours (CNS; n=13), sarcomas (n=8), neuroblastomas (n=1) and other solid tumours (n=4). We performed ~90X whole genome sequencing on these tumours and corresponding germline samples (at ~30X) and analysed these data using computational genomic tools, including SAGE, PURPLE and LINX to identify somatic single nucleotide variants (SNV), copy number variants (CNV) and structural variants. We then applied SNV based tumour evolution framework to identify clonal and subclonal populations. The order of mutation clusters was inferred using evolutionary principles. We also identified four tumour samples that have undergone whole-genome doubling (WGD). The timing of WGD was inferred using GRITIC pipeline.
Results: In this cohort of NTRK-fusion tumours, SNV based clonality analysis revealed that 23 out of the 26 tumours were polyclonal in nature, while the monoclonal tumours exhibited low purity. Multi-time point analyses from two patients showed evidence of branching evolution. One CNS tumour presented with founding whole genome doubling event, while in other tumours WGD was relatively late event.
Conclusion: Polyclonality is a common feature of NTRK-fusion paediatric tumours, while timing of WGD may explain evolution of some tumours. Such inherent features of clonal evolution may help delineate therapy resistance in these tumours.

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CBL mutations in CNS and solid tumours - a new therapeutic opportunity?

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Background: Molecularly targeted therapies improve outcomes for childhood cancer patients and are made possible by personalised medicine approaches like the Zero Childhood Cancer Program (ZERO). Mutations in CBL, an E3 ubiquitin ligase, represent one such molecular target in acute myeloid leukaemia, where CBL mutation mediates activation of the Receptor Tyrosine Kinase (RTK) FLT3 and sensitivity to FLT3-targeted Tyrosine Kinase Inhibitors (TKIs). We have identified novel CBL mutations in paediatric CNS and solid tumours, raising the possibility that CBL mutations mediate RTK activation in other tumour types and these patients may benefit from TKI therapy.
Methods: We analysed the ZERO cohort for somatic mutations in CBL. Molecular (whole genome, RNA sequencing and methylation) and high-throughput drug screening data from CBL mutated-patients, performed as part of ZERO, were interrogated. In silico pathogenicity analysis (ESM-2) was performed on novel variants. CBL mutations were cloned from patients and modelled in vitro to analyse the functional and signalling impacts of CBL mutation.
Results: We identified 26 somatic CBL variants in 22 individual patients, including 14 patients with CNS tumours. Thirteen non-haematological tumour samples harboured CBL mutations that are established drivers (exon 8/9Δ) or suspected to be oncogenic given their location in critical domains and in silico pathogenicity analysis. Over half of these samples had molecular indications of RTK activation (7/13). Most CNS tumours did not fall into a pre-defined DNA methylation-based subtype classification, suggesting CBL mutation may represent a distinct molecular entity. In vitro modelling of exon 8/9Δ in a neuroblastoma cell line demonstrated that this variant enhances proliferation through RTK activation in other tumour types.
Conclusions: CBL mutation may be a marker of RTK activation and therapeutic target in CNS and solid tumours. This finding will extend the benefit of TKIs, which have proven efficacy in paediatric cancer, to more patients.

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Identifying non-coding lncRNA dependencies in high-risk MLL-rearranged acute myeloid leukaemia

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Background: CRISPR-Cas9 genome-wide screening has comprehensively mapped the dependency landscape in cancer, including paediatric subtypes. Whilst therapeutic discovery in certain cancers has benefitted from this, other high-risk subtypes, such as MLL-rearranged acute myeloid leukaemia (MLLr-AML), still lack therapeutic options. Existing work in the dependency-mapping space has focused predominantly on the coding genome, ignoring potential novel cancer dependencies in non-coding regions. Long non-coding RNAs (lncRNAs) in particular represent untranslated RNAs that are increasingly implicated in cancer development, including in MLLr-AML. In the current study, we perform both CRISPR-Cas9 screening and Cas13d secondary screening to identify novel lncRNA dependencies in MLLr-AML, with Cas13d as a validation tool that better emulates therapeutic conditions, where genes are often modulated instead of completely suppressed.
Methods: Stable Cas9-positive MLLr-AML cells were generated using lentiviral transduction, with enzymatic activity confirmed through single-target knockdown. In vitro genome-wide CRISPR-Cas9 lncRNA KO screening was performed in three Cas9-positive MLLr-AML cell lines transduced with single guide RNA (sgRNA) libraries at ~500x coverage. Following 21 days in culture, gDNA was isolated and sequenced to observe overall sgRNA representation. lncRNA dependencies were identified using both MAGeCK and CHRONOS analysis softwares. Stable Cas13d-positive MLLr-AML cells were similarly generated for pooled CRISPR-Cas13d secondary screening.
Results: MAGECK identified 5 lncRNA gene dependencies (fold-change < -1.5, FDR < 0.01) overlapping all three cell lines in the CRISPR-Cas9 screen. In CHRONOS, 18 genes showed strong dependency (gene-effect score < -1) across all three cell lines. A custom Cas13d library for secondary screening was designed containing sgRNAs targeting the identified dependencies. Three stable Cas13d-positive MLLr-AML cell lines were generated in preparation. Flow cytometry following transduction with CD45 sgRNAs showed successful knockdown in these lines.
Conclusion: Utilising a genome-wide screening approach we have identified lncRNA dependencies in MLLr-AML which will be further validated utilising a CRISPR-Cas13d secondary screening strategy.

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Individualized-tumor-informed panel approach enables ultra-sensitive ctDNA-based minimal disease monitoring for pediatric cancers

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While the use of ctDNA-based minimal residual disease (MRD) monitoring is gaining popularity in adult cancers, this approach is not yet widely adopted in paediatric cancers (PCs). However, this approach is not widely implemented in pediatric cancers. Enabled by the ZERO program, Australian's national Childhood Cancer Precision Medicine program, we developed a robust and sensitive detection method to monitor ctDNA in PCs. This method overcomes PC-specific challenges including low tumor mutational burden, lacking of hotspot mutations and limited blood from children. Specifically, our assay includes personalized panel design which targets primary tumor mutations from each patient, a fixed panel targeting hotspot mutations, error suppression from duplex deep sequencing and customized software for UMI de-duplexing (REDUX) and tumor fraction estimation (WISP).
We retrospectively analyzed 661 blood or cerebrospinal fluid (CSF) samples from 168 PC patients. We achieved an average detection limit of 0.005% ctDNA fraction and a minimal limit of 0.0001%. We detected ctDNA burdens ranged widely from 0.001% to 100% with brain (CNS) tumors consistently showing a low ctDNA burden (average 0.03%). When patients were identified with high disease burden, our approach identified ctDNA in over 93% of extra-cranial solid tumours, 90% of leukaemia, 89% of sarcoma, and 90% of Neuroblastoma. Our approach showed that ctDNA burden dynamics correlated with disease progression or remission. Importantly, we identified circulating disease in ~20% of clinically complete response assessments, predicting relapse 1–6 months before clinical manifestation. Finally, with the fixed panel, our assay identified emergence of novel variants, suggesting potential new treatment options during tumour evolution. Overall, these results demonstrated that our ctDNA-based disease monitoring approach for pediatric cancer is an

important addition to standard of care, quantifying disease burden and tracking disease evolution for precision-guided treatment.

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A Data Lakehouse for Childhood Cancer Precision Medicine

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Background: The Zero Childhood Cancer Program (ZERO) is a precision medicine program with currently ~1600 patients enrolled to date, with the program forecasted to have 3500 patients by end of 2025. This poses two data challenges: how to store and analyse billions of germline variants from this cohort, and how to integrate different data sources and types from within and outside the program to interpret analysis outcomes. For these large and complex datasets, we selected a Data Lakehouse architecture for its combination of performance, data-type flexibility and cost-effectiveness.
Method: To address these challenges, we implemented a cloud Data Lakehouse underpinned by the Delta file format and distributed computation engine Spark, on the Databricks platform. Additionally, we created a scalable data pipeline to investigate pharmacogenic interactions with adverse drug reaction (ADR) in ZERO patients to demonstrate the ability to efficiently integrate heterogenous data.
Results: We successfully populated a distributed germline variant database with >1600 whole genomes for query and analysis; this achieved genome range cohort queries for over 8.3 billion variants in under 5 seconds and a 60:1 compression ratio from raw VCF (10TB to 165GB).
We then integrated PharmKGB's drug-pharmacogenic interactions and ZERO's ADR dataset with ZERO's germline database, enabling detection of patients with ADR patients that had pharmacogene alleles related to their ADR-causing drugs. We identified 3 ADR patients out of 34 patient-drug combinations with possible pharmacogene links, one having a well-documented pharmacogene (UGT1A1*93) related to their ADR therapy.
Conclusion: This project demonstrates how the Data Lakehouse architecture can be successfully implemented to meet the scale of the ZERO program's analytical needs.

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PREDICT and Validate: Investigating Germline Alterations in Paediatric Cancer Predisposition

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Germline alterations in cancer predisposition genes (CPGs) are associated with increased risk of developing certain cancers. Identifying underlying germline genetic contribution to cancer development not only assists in developing a personalised management plan for the patient, but also provides cancer risk clarification and guidance for family. Studies suggest 10-15% of paediatric cancer patients carry germline pathogenic variants in CPGs. However, a proportion of patients with features suggestive of underlying cancer predisposition, have no pathogenic alterations identified in known CPGs. The PREDICT study was conducted to identify germline variants in patients <21 years, newly diagnosed with cancer across NSW, together with biological parents where possible (trio analysis), using whole genome sequencing (WGS). Our team aimed to comprehensively interrogate the genome sequencing data to identify and functionally validate novel germline variants that may contribute to cancer predisposition.

We have established bioinformatics pipelines to analyse PREDICT germline WGS data against a panel of ~1250 cancer-related genes, to investigate genomic alterations within and beyond protein-coding regions, including exonic single nucleotide variants and small insertions/deletions, splicing variants, non-coding region variants (e.g. promoter regions), and structural alterations. Trio-based WGS data was run on The University of Sydney's High Performance Computing system, with analysis incorporating RStudio and online curation tools.

Clinically actionable pathogenic/likely pathogenic variants were detected in 51/230 (22.2%) enrolled patients (e.g. PMS2, NF1), and 62 novel candidate variants were identified in 53/230 (23.0%).

Functional validation of prioritised variants-of-interest recurrently identified in B cell leukaemia/lymphoma, is currently being conducted in transduced murine pro-B cell line Ba/F3, which stably express wildtype and each mutant, to assess their functional impacts on the relevant pathway. Our comprehensive WGS analysis has identified known and novel germline alterations. Further characterisation of novel findings may broaden our understanding regarding underlying mechanisms of paediatric cancer predisposition, and lead to newly defined genotype-phenotype associations.

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A collaborative implementation science approach to genomics and precision medicine multidisciplinary teams

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Background: Rapid advances in genomics and precision medicine have led to increased demand for genomics expertise, and access to gene therapies. To address this, the Sydney Health Partners Clinical Academic Group (CAG) in Genomics and Precision Medicine uses implementation science to address the barriers and gaps that patients, clinicians, and health systems face in this field.
A key barrier is how non-genetics health professionals can engage with genetics and genomics expertise to enable genetic diagnosis and management. In our systematic review, a genomic multidisciplinary team (MDT) approach was valuable, however, barriers remain in sustainability and reach.
Aim: The aim of this study was to investigate impact of genomic MDTs at SCHN-Westmead, as an exemplar of this approach, with applications to additional hospitals and subspecialties. These MDT meetings include clinical genetic, laboratory, and subspecialist staff in paediatrics, neurology, oncology and ophthalmology.
Methods: Cases and clinical outcomes across genomic MDTs were recorded. The Reach Effectiveness Adoption Implementation (REAIM) framework was used to study genomic MDTs between 2020-2022. A survey of MDT attendees was designed based on the Theoretical Domains Framework (TDF) to study impacts on behaviour change.
Results: Genomic MDTs at SCHN-Westmead, saw over 1000 cases across 150 MDT meetings in over 8 subspecialties, For the general paediatric genomic MDT, 205 patients across 34 meetings facilitated 124 genomic tests, of which 39% received a genetic diagnosis. There was broad adoption across paediatric departments, including rural clinicians and 'mainstreaming' of genomics. Surveys of 33 clinician attendees demonstrated the MDT improved key TDF domains such as knowledge, skills, and beliefs about capabilities, and was a key influence on mainstreaming.
Conclusion: Overall the genomic MDTs facilitated rapid genomic diagnosis, upskilling of healthcare professionals, and improved uptake of genomics. We will continue to explore how to improve uptake, across additional areas of medicine in our CAG.

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Steps to resolution of variants of uncertain significance in RPE65

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Background: Early-onset severe retinal dystrophy and Leber congenital amaurosis lead to marked visual impairment in childhood and progressive visual loss. These conditions may be caused by variants in the RPE65 gene, which encodes an isomerisation enzyme found in retinal pigment epithelial cells (RPE). Affected patients with bi-allelic, pathological variants in this gene may benefit from the sight-saving RPE65 gene therapy, voretigene neparvovec-rzyl (Luxturna, Novartis). However, approximately a third of the variants reported in RPE65 in the international variant database, ClinVar, are classified as variants of uncertain significance (VUS), rendering patients ineligible for therapy. Functional assessment of these variants is crucial for therapy eligibility. In this study we are investigating biomarkers, including gene/protein expression and high pressure liquid chromatography (HPLC) in induced pluripotent stem cell-derived RPE (iRPE), to aid variant interpretation and therapy eligibility.
Methods: CRISPR-Cas9 gene editing technology was used on normal iPSCs to introduce missense variants in RPE65. Cells containing normal or missense variants in RPE65 were expanded and differentiated. RPE65 transcript and protein expression levels were validated through RT-qPCR and western blot respectively. In addition, steps were undertaken to optimise the HPLC methodology using retinoid standards.
Results: We have found that iRPE containing RPE65 missense variants may lead to reduced expression of RPE65 both at the RNA and protein level, compared to normal control. We have also optimised the HPLC methodology for accurately detecting the various retinoids associated with RPE65 function.
Conclusion: We have optimised methods to detect functional differences in isomerisation capability of normal RPE65 compared with RPE65 containing missense variants. This is a crucial stepping stone in reclassifying RPE65 variants in patients, enabling them an opportunity for access to sight-saving gene therapy.

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Genetic editing applications for variant classification and therapy development for cone-rod dystrophies

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Background & Aims: Inherited retinal dystrophies (IRDs) affect around 1 in 1,000 individuals and frequently result in progressive degeneration of retinal cells, and eventually blindness. Variants occurring in genes and proteins, such as ABCA4, PROM1 and RPGR, affect photoreceptors and retinal pigment epithelium (RPE) cells and cause IRDs, such as Cone-Rod Dystrophies (CRD). Two major concerns in CRD include classification of variants of uncertain significance (VUS) and current unavailability of therapies for recurrent variants. The aim of this study is to characterise and reclassify VUS in CRD-causing genes and to design novel therapy approaches for recurrent variants. Both aims will be achieved using genetic editing in vitro with reference to the American College of Medical Genetics and Genomics (ACMG) criteria for variant analysis.
Methods: For VUS investigation, known pathogenic, benign and uncertain variants were introduced into control human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas-9 Homology-Directed Repair (HDR) and were then differentiated into retinal tissues, with a particular focus on the RPE. For therapy development, a prime editing strategy was designed and investigated to assess correction efficiency. Additional controls and patient-derived hiPSCs were used for comparisons in both aims.
Results: Using CRISPR/Cas-9 HDR, six hiPSC lines were created each containing a VUS, pathogenic or benign variant. Microarray and sequencing studies confirmed no off-targeting events in these lines prior to use in subsequent investigation. Multiple patient-derived hiPSCs differentiated into RPE revealed reduced subject protein expression when compared to control. CRISPR/Cas-9 HDR in HEK293Ts successfully introduced a recurrent variant for therapy development. Prime editing plasmids were designed to test correction efficiency in HEK293T disease model in preparation for application in patient-derived hiPSCs.
Conclusions: Genetic editing approaches are useful for reclassification of VUS and novel therapy development targeting recurrent CRD-variants. Optimisation of this workflow will be valuable and applicable for other IRDs.

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Estimating population prevalence of rare diseases in children using administrative health data

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Background: Although around 8% of the population is estimated to have a rare disease, a major challenge is accurately identifying these individuals in the health system. The true prevalence in Australia is currently unknown. While Orphanet provides a classification system for rare diseases, this has not been applied in NSW. We aimed to apply Orphanet classification to NSW administrative health data to investigate the prevalence of paediatric rare diseases.
Methods: We conducted an NSW-wide population-based study of all children <18 years admitted to hospital who received a rare disease code between 2010-2019. Data were ascertained from the NSW Admitted Patient Data Collection where diagnoses associated with each admission are coded using the International Classification of Disease version 10-Australian Modification (ICD10-AM). We mapped 6,207 ORPHA codes to 3,230 ICD10-AM codes using the 2023 Orphanet Masterfile and Walker et al. (2017) resource to classify rare diseases in our data, and aggregated these to 31 primary medical categories. Overall frequency and rate in 2018 by major categories and by sex and age-group were determined.
Results: 300,127/ 1,605,491 (18.7%) children admitted to a NSW hospital in 2010-2019 had a rare disease code, 55% were male with mean age of 5.1 years (SD 5.7 years). The three most common categories were 'rare neurologic diseases' (19.5%), 'rare developmental defects during embryogenesis' (19.3%) and 'rare respiratory diseases' (9.7%). Prevalence of rare diseases requiring hospitalisation in 2018 was 2.3%; rates ranging between 0.001-0.778%.
Conclusion: We have developed a contemporary coding set to map Orphanet codes to ICD10-AM and demonstrated its application to enable use of administrative health data to calculate population prevalence. With linked data, this approach provides the potential to track cross-sectoral outcomes of rare diseases, critical for planning appropriate models of care for these collectively common and impactful conditions, and assess impact of precision medicine initiatives.

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Systematic review of population-based studies investigating rare diseases in children

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Background: Rare diseases are estimated to affect 6-8% of the world's population. Few population-based studies examine rare diseases as a collective, instead focusing on specific rare diseases. The aim of this study was to systematically review, identify and evaluate the methods utilised and prevalence estimated by population-based studies on rare diseases in children.
Methods: A search of MEDLINE via Ovid was conducted from January 2014 to June 2024 for population-based studies of rare diseases. Titles and abstracts were screened for population-based studies, published in English, focused on more than one type and/or category of rare disease. Supplementary reference/citation search was also conducted to identify further relevant studies. Two reviewers screened full-text articles for inclusion and performed data extraction. Information on study setting, time-period, data sources, study population (paediatric), classification, measures and main findings were extracted.
Results: 15 population-based studies met the inclusion criteria. Studies identified rare diseases using two main methods, via: 1) a population registry of patients with rare diseases, or 2) administrative health data sources such as hospital admission or mortality data using ICD10 or Orphanet diagnostic codes to identify patients with rare diseases. Studies examined different measures of rare diseases including population prevalence, proportion of the study cohort affected by a rare disease, mortality and hospital length of stay. Of the three studies that examined prevalence of rare diseases as a group, prevalence ranged between 1.5-2.3% of the population.
Conclusion: There are few population-based studies examining prevalence of rare diseases as a group, and of these, different sources, measures and outcomes of rare disease were assessed. Standardised classification of rare diseases using administrative health data would facilitate more population-based studies to enable investigators to examine real-world epidemiological data for rare diseases.

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AAV-mediated DARS1 gene replacement in preclinical models of the leukodystrophy HBSL

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Leukodystrophies are a group of heritable diseases affecting the development and maintenance of white matter within the central nervous system (CNS). Hypomyelination with Brainstem and Spinal cord involvement and Leg spasticity (HBSL) is a currently untreatable leukodystrophy that leads to severe physical disability, neurological abnormalities, and shortened life expectancy. HBSL is caused by biallelic loss-of-function mutations in the DARS1 gene, which encodes the cytosolic aspartyl-tRNA synthetase; an enzyme essential for protein synthesis. Here, we have developed and tested adeno-associated virus (AAV)-mediated DARS1 gene replacement to establish proof-of-concept for the treatment of HBSL.

We have generated two novel mouse models of DARS1 deficiency within the CNS, through conditional knockout of Dars1 in oligodendrocytes (Dars1OligoKO) and neurons (Dars1NeuroKO) of C57Bl/6J mice, using the Cre-loxP system. Concurrently, we have developed AAV vectors for targeted delivery of functional human DARS1. To maximise CNS transduction in this proof-of-principle study, we have selected the AAV.PHPeB capsid serotype. Our AAV gene expression cassette includes a codon-optimised and cytosine-phosphate-guanine (CpG) motif-free DARS1 coding sequence to promote strong, safe and long-term transgene expression.

Both Dars1OligoKO and Dars1NeuroKO mice exhibit pronounced pathological phenotypes that resemble key aspects of HBSL, including neurodegeneration, motor dysfunction, and reduced survival. Systemic delivery of our optimised AAV.DARS1 vector has shown significant efficacy in rescuing the phenotype of our HBSL model mice.

Our results provide proof-of-concept for an AAV-based DARS1 gene replacement therapy for HBSL. More broadly, our targeted gene delivery and disease modelling approaches will guide the development of AAV-mediated gene therapies for other leukodystrophies and monogenic diseases of the CNS.

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Preclinical assessment of gene therapy for RARS2-related early onset epileptic encephalopathy

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Background: Developmental and epileptic encephalopathy (DEE) caused by biallelic RARS2 pathogenic variants is a rare neurological disorder that impairs cognitive and motor functions. RARS2 encodes the mitochondrial arginyl-tRNA synthetase protein which is essential for mitochondrial protein synthesis. Variants in RARS2 disrupt normal brain development, leading to early-onset epileptic encephalopathy where symptoms typically manifest in infancy, characterised by drug-resistant epilepsy, developmental delay, and profound intellectual disability. Understanding the molecular and cellular mechanisms underlying RARS2-related DEE remains challenging due to the scarcity of brain tissue samples and suitable animal models. In this project, we utilise human cortical brain organoids derived from induced pluripotent stem cells (iPSCs) to study RARS2-related DEE at the cellular and molecular level. Importantly we aim to establish proof of concept for AAV-RARS2 gene therapy as a potential therapeutic approach for this disease.
Methods: We used homology-directed repair CRISPR gene editing to generate an iPSC line with a RARS2 pathogenic variant (c.392T>G; p.(Phe131Cys)). These iPSCs were differentiated into dorsal cortical organoids to model RARS2-related DEE. The cortical organoids were cultured in suspension for several months to develop cortical layers and complex neuronal networks. An electrophysiology-relevant medium was introduced at 100 days in vitro to better support synaptic activity and neuronal function.
Results: Multi-electrode array recordings were performed in day 150 organoids revealing that RARS2-variant organoids exhibited higher neural activity compared to isogenic controls. Preliminary validation of the RARS2 gene therapy construct demonstrated an increase in RARS2 gene expression and protein.

Conclusion: These data demonstrated that brain organoids are suitable model systems to study neurodevelopmental disorders providing novel insights into network functionality in disease. Furthermore, this project will provide a pipeline for developing gene therapies for other mitochondrial disorders.

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PRECISE – Co-designing a genomics and precision medicine program for primary care

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Background: Precision medicine and the growing demand for genomics in all areas of medicine has led to massive challenges in service provision, education and upskilling for non-genetics healthcare professionals. This is particularly an issue in primary care, where genomics and precision medicine is increasingly seen as standard healthcare. GPs are able to order genomic tests such as in non-invasive prenatal screening, reproductive carrier testing, and are increasingly seeing patients with genetic conditions. However, there has been relatively slow uptake of genomics in primary care, with practitioners reporting inadequate capacity, training, and support to provide genomic care to patients.
The PRECISE (Practitioner Readiness, Education and Capabilities, with Implementation Science Evaluation) Project is a MRFF funded research program that will build capacity in genomics for primary care practitioners and evidence-based strategies for further implementation into the primary health care sector.
Methods: This project brings together a multidisciplinary team comprising of consumers, primary healthcare, academia, industry, decision makers and genetics services. It will utilise implementation science frameworks, as well as principles of education co-design, and program evaluation with stakeholders and consumers. This project aims to both co-design education resources and identify strategies to improve capacity within the primary healthcare sector. This is with an implementation science approach incorporating the Knowledge to Action cycle.
Results: Our initial scoping review has already identified key attitudes, needs, and gaps in genomic education in the sector. This will inform our co-design stage, to produce resources to support primary care practitioners in applying genomics, as well as knowledge on strategies to improve clinical capacity in the sector.
Conclusion: The PRECISE team is a unique and collaborative approach, bringing a diverse team of consumers, clinical genetics, postgraduate education, implementation science and evaluation, public health, primary care, industry and policy with the aim to build genomics capabilities in this sector.

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Newborn Screening for Duchenne muscular dystrophy: Development of a scoping study

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Duchenne muscular dystrophy (DMD) affects approximately 1 in 5000 live male births worldwide and is characterised by progressive muscle weakness and atrophy. Several dystrophin restoration therapies have been developed and serve as a promising therapeutic approach for DMD, however potential benefits of earlier treatment or intervention prior to disease onset are not well understood. Newborn Genomic Sequencing in Screening: Therapy Ready and Information for Life (TRAIL) TRAIL study has set out to investigate the potential use of genomic technologies to expand newborn screening (NBS) capabilities. The study aims to conduct the first DMD NBS scoping study in New South Wales (NSW) and the Australian Capital Territory (ACT) to evaluate the feasibility, acceptability, and benefit of screening newborns for DMD. The scoping study will run for 12-months, screening 100,000 newborns across NSW and ACT using dried blood spot (DBS) cards. The first-tier screen will measure muscle-specific creatine kinase isoform (CK-MM) concentrations. Rapid aneuploidy detection by QF-PCR will be performed on the cards with raised CK-MM levels to identify males. Third-tier screen will identify pathogenic variants in the DMD gene using a massively parallel sequencing capture panel on male screen positive cards. Newborns with pathogenic DMD variants will be referred for diagnostic testing and the model of care will be assessed for newborns diagnosed through the scoping study. The study findings will help inform future scoping studies and model of

care approaches for other genetic conditions in the trajectory of genomic NBS and implementation into health practice and policy.

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From recommendation to access: Improving procurement of precision-guided treatments with implementation science

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Background: ProCure is a novel online, interactive paediatric oncology medicines access database co-designed with healthcare providers (n=17, paediatric oncologists, nurse consultants and allied health professionals) and developed using implementation science methods to streamline the application process for accessing targeted therapies. Following beta testing with end users (n=12, paediatric oncologists, pharmacists and scientists) in September 2023, ProCure was found to an acceptable and appropriate resource. These findings combined with its co-design indicate that ProCure is ready for implementation. This study aims to assess its implementation and effectiveness at paediatric centres nationally using a two-arm, parallel, randomised cluster trial with a type 3 effectiveness-implementation hybrid design.
Methods: A focus group will co-design an implementation support package (ISP) through consultation with clinicians from each paediatric centre. The ISP will be based on targeted, evidence-based strategies informed by the Expert Recommendations for Implementing Change tool, to mitigate barriers previously identified using the Consolidated Framework for Implementation Research. ProCure will be delivered to all sites, but only one trial arm will receive the ISP. ProCure's implementation outcomes and clinical effectiveness will be measured through inter-arm and pre- and post-trial comparisons of data collected from surveys, health system audit data, web metrics, and interviews. These analyses will be conducted separately for high-risk and non-high-risk patients.
Results: Higher adoption and sustained implementation of ProCure in the intervention arm would indicate the ISP's effectiveness. Increased confidence among clinicians, along with a reduction in the time required to complete and submit compassionate access applications would demonstrate ProCure's effectiveness as a service intervention.
Conclusion: Outcomes from this implementation trial will provide further insight into clinicians' compassionate access of precision-guided treatments recommended by precision medicine trials. ProCure's role as an intervention to facilitate uptake of precision oncology recommendations in a clinical context will inform future international scale-up of ProCure.

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Co-developing positive psychology resources with caregivers of children with developmental epileptic encephalopathies

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Developmental and epileptic encephalopathies (DEEs) are a group of life-threatening and life-limiting conditions characterised by early-onset intractable seizures, debilitating co-morbidities, and premature mortality. Extensive progress has been made in understanding DEE pathophysiology: over 400 genes have been reported, with a growing number of genetic identifiers still being discovered. However, advances in gene diagnostics have yet to translate into precision therapies for affected children and the psychosocial resources to support families are limited. This research used a mixed-methods, multi-perspective approach to investigate caregiver and family psychosocial experiences undergoing genetic testing for DEE, to co-develop a suite of tailored psychosocial supports.
Phase one involved a systematic literature review of the international literature to identify caregivers' information needs and preferences to support clinical practice. In phase two, I conducted a mixed-methods interview study investigating caregivers' experiences undergoing genetic testing, to identify their psychosocial support needs, before, during and after their child's genetic testing. In phase three I convened a priority-setting project, engaging caregivers and multidisciplinary healthcare professionals to codesign a suite of psychological resources using a person-based approach. Based on the findings of phase one to three, I developed 'Finding a Way', a suite of audio-visual psychological resources tailored to support psychosocial adjustment to a child's genetic DEE diagnosis. In stage four, I conducted a mixed-methods study to evaluate the acceptability and emotional impact of 'Finding a Way' among an international cohort of caregivers of children with DEEs. Our qualitative and quantitative data provided evidence that suggested personalized positive psychology resources ('Finding a Way') can enhance caregiver emotional adaptation to their child's genetic DEE diagnosis.
This translational program of work delivered high-quality, innovative psychological resources tailored to address specific psychosocial challenges experienced among caregivers of children with genetic DEEs.

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A pilot study of creating a long-term follow-up clinical registry and biorepository of patients undergoing cell and gene therapies

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Background: Emerging treatments such as cell and gene therapies (CGT) offer new possibilities for treating challenging medical conditions with limited or no treatment options, from rare inherited disorders to certain forms of cancer. These therapies aim to achieve therapeutic effects through long-lasting or permanent changes in the human body. However, their interaction at the cellular and genetic levels carries risks, such as delayed adverse events and uncertain long-term benefits. Therefore, extended monitoring, or long-term follow-up (LTFU), is essential for safety, efficacy, treatment durability, understanding late-onset effects, regulatory compliance, future research, and ensuring patient well-being.
Methods: Our pilot will explore the collection and storage of data from multiple sources and biospecimens in the NSW Health Statewide Biobank, as well as the process of data linkage for long-term follow-up of patients receiving CGT products. Our platform will facilitate the extraction of data from medical records, including real-world data and patient-reported outcomes, and enable linkage with other existing databases. This process will be supported by transparent and consistent data governance practices.
Results: The study is in the planning phase and is actively seeking investigator-initiated clinical trials in CGT to support the development of a long-term follow-up database for CGT products. This pilot study would serve to demonstrate NSW's ongoing leadership in gene and cell therapy, as well as emerging leadership capabilities in new registry models
Conclusion: Creating an accessible LTFU database and biorepository with data linkage for CGT products could enhance understanding of these therapies' safety and effectiveness over time, benefiting patients, clinicians, researchers, industry, and policymakers. Storing biospecimens in the NSW Health Statewide Biobank could also support research on under-studied diseases, like childhood dementia, and assist in monitoring these therapies.

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Gene therapy proof-of-concept for spastic paraplegia 56

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Hereditary spastic paraplegias (HSP) are a group of neurological disorders characterized by progressive lower limb weakness and spasticity; with a global prevalence of 3.6 in 1000 people. SPG56 is a subtype of HSP caused by autosomal recessive mutations of the CYP2U1 gene, which encodes CYP2U1, a cytochrome P450 hydroxylase predominantly found in cells of the central nervous system (CNS) and thymus. To date, no curative treatments exist. This study tests the efficacy of adeno-associated virus (AAV)-mediated CYP2U1 gene replacement in a Cyp2u1-/- knockout mouse model to establish the proof-of-concept for SPG56 gene therapy.
AAV9 was used for effective CNS transduction paired with either the CAG (AAV9.CAG.hCYP2U1) or EF1a promoter (AAV9.EF1a.hCYP2U1) for strong and moderate CYP2U1 expression, respectively. Cyp2u1-/- knockout mice were treated via intravenous or intracerebroventricular injections. Controls included untreated knockout, heterozygous and wild-type mice. Behavioural tests assessed motor function and learning, whilst blood samples were analysed for serum biomarker levels (CoQ9 and CoQ10). Post-mortem biochemical and histological analyses were performed to evaluate vector distribution and transgene expression.
First, the validity of the Cyp2u1-/- mouse strain as an SPG56 model was established. Serum CoQ9 and CoQ10 levels were elevated in knockout mice compared to wild-type and heterozygous controls, consistent with human SPG56 patients. Cyp2u1-/- mice showed sensorimotor gating deficits, indicated by reduced pre-pulse inhibition of the acoustic startle response, along with learning deficits on the y-maze and passive avoidance tests. Next, preliminary results of AAV-mediated CYP2U1 gene replacement showed significantly lower CoQ9 and CoQ10 levels and depicted trends towards normalisation of behavioural impairments.
This study provides the proof-of-concept for CYP2U1 gene replacement as an effective treatment for HSP SPG56, which paves the way for further development and clinical translation of this therapy – holding great promise to permanently cure this debilitating condition.