The Academy of Medical Sciences

Clinical Academics in Training Annual Conference 2022

Thursday 19th May 2022

Abstract booklet

Clinical Academics in Training Annual Conference 2022 (CATAC)

Thursday 19th May 2022

Contents

The Academy of Medical Sciences

The Academy of Medical Sciences is the independent body in the UK representing the diversity of medical science. Our mission is to promote medical science and its translation into benefits for society. The Academy's elected Fellows are the United Kingdom's leading medical scientists from hospitals, academia, industry and the public service. We work with them to promote excellence, influence policy to improve health and wealth, nurture the next generation of medical researchers, link academia, industry and the NHS, seize international opportunities and encourage dialogue about the medical sciences.

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Welcome to CATAC 2022

'I am delighted to welcome you to the Clinical Academics in Training Annual Conference 2022. The last two years have been a challenging time for the clinical community, and it is fantastic to be able to bring everyone together once again. I am greatly looking forward to showcasing the incredible breadth of work that the Academy undertakes with a very diverse community of researchers.

Please join me in thanking Professor John Iredale FRSE FMedSci, and Professor Jane Norman FMedSci for hosting today's meeting and Professor Charlotte Summers for delivering what I'm sure will be a wonderful Keynote Speech. I urge you all to learn more about the Academy and to take full advantage of today's event to meet fellow researchers across all career stages, learn about cutting edge research within academic medicine, and share your own research with the wider community.'

Professor Dame Anne Johnson DBE PMedSci, University College London

'For the last few years, clinical academics have been working through incredibly challenging times. After many months of virtual meetings, it's more important than ever for researchers to come together and share our work, so I am delighted that CATAC is able to go ahead in person this year!

As well as celebrating your research achievements, this conference is a wonderful opportunity to develop new ideas and partnerships for the future. I look forward to meeting in Bristol, and hope you will enjoy this chance to reconnect with the clinical research community and learn about each other's fascinating work.'

Professor John Iredale FRSE FMedSci, University of Bristol

'I am delighted to once again be a part of this prestigious event. As well as chairing proceedings today, I have also had the honour of helping to select the abstracts that make up our fantastic programme. The standard of abstracts received this year was exceptionally high, and it was certainly a tough job to narrow them down. This standard illustrates the value of hosting meetings like CATAC outside of London, and I greatly look forward to hearing some of the brilliant research that is being done. I'd like to encourage fellows and junior colleagues present to really get involved, to make new connections and to learn something new.'

Professor Jane Norman FMedSci, University of Bristol

Keynote speaker

Professor Charlotte Summers

Professor of Intensive Care Medicine and Director of Clinical Academic Training, University of Cambridge

'I am delighted to be asked to deliver this year's Keynote Speech at the Academy's Clinical Academics in Training Annual Conference. After two years of staying apart due to the COVID-19 pandemic, in which the impact on clinical academics was unprecedented, it's now more important than ever that we have the opportunity to get together, share our experiences, forge new relationships and support one-another as we move forward into the next stages of our careers.

As Professor of Intensive Care Medicine and Director of Clinical Academic Training, I wholeheartedly value the importance of clinical research; taking basic science and transforming it into therapies to improve human health. My career journey to-date has been interwoven with support from the Academy, with grant funding via the Starter Grants for Clinical Lecturers awards and career development support through their flagship leadership programme, FLIER. I'm pleased to have this opportunity to share my experiences with clinical researchers, particularly those at the beginning of their careers. I hope that this meeting will be a valuable opportunity for them to discover more about pursuing a career in clinical medicine and will inspire them in their journey to become the research leaders of the future.'

Charlotte Summers is the University Professor of Intensive Care Medicine. Charlotte graduated in both Biomedical Sciences and Medicine from the University of Southampton, and later undertook a PhD at the University of Cambridge, alongside specialist clinical training in Respiratory (Cambridge) and Intensive Care Medicine (London). Subsequently, Charlotte was appointed as the UK's first NIHR Clinical Lecturer in Intensive Care Medicine, and awarded both a Fulbright All-disciplines Scholar Award and a Wellcome Trust Fellowship for Postdoctoral Clinician Scientists to undertake research at the University of California, San Francisco. Charlotte returned to Cambridge in 2015, where she currently co-leads the Peri-operative, Acute, Critical Care and Emergency medicine (PACE) Section of the Department of Medicine alongside being the Clinical School's Director of Clinical Academic Training.

Charlotte is a member of the UK-COVID Therapeutic Advisory Panel and Chief Investigator of HEAL-COVID, the national Urgent Public Health platform clinical trial that is aiming to find drug therapies to improve the longer-term clinical outcomes of people who were hospitalised with COVID-19.

Programme

Bristol Harbour Hotel, 53-55 Corn St, Bristol BS1 1HT

09:00 **Registration and poster setup**

09:30 **Welcome**
Professor John Iredale FRSE FMedSci

09:45 **Post-Doctoral Plenary Competition**
Each competitor will have ten minutes to present their research, followed by five minutes for questions

'Non-communicable disease, sociodemographic factors, and risk of death from infection: a prospective cohort study of 493,295 UK Biobank participants' - Dr Michael Drozd, University of Leeds and Leeds Teaching Hospitals NHS Trust

'Evidence that the Ser192Tyr/Arg402Gln in *cis* tyrosinase gene haplotype is a disease-causing allele in oculocutaneous albinism type 1B (OCA1B)' - Dr Siying Lin, Torbay and South Devon NHS Foundation Trust and Royal Devon & Exeter NHS Foundation Trust

'Socioeconomic support to prevent tuberculosis among household contacts of people with tuberculosis in Peru: a community-randomised controlled trial' - Dr Matthew Saunders, London School of Hygiene and Tropical Medicine and Royal Free London NHS Foundation Trust

'Harnessing liver-resident gamma delta T cells for immunotherapy of hepatocellular carcinoma' - Dr Nekisa Zakeri, University College London

10:45 **Refreshment break**

11:00 **Poster competition**
Each poster presenter will have two minutes to present their research to a panel of judges. Please feel free to join a group of judges or to make your own way around the poster exhibition. Posters are grouped by four broad research categories.

Group A: Applied health services research; Epidemiology; Population Health Sciences
Group B: Cellular and molecular biology; Genetics
Group C: Inflammation; Infection; Immunity
Group D: Neuroscience; Imaging; Technology

12:00 **Lunch break**

13:00 **Panel session with networking**

Transdisciplinary research and cross-sector partnerships at the NHS-academia interface

Please join our session Chair and panellists in a discussion about the benefits and challenges of forging new partnerships across research disciplines and sectors, for the enhancement of your research career and to improve healthcare research and delivery. There will be opportunities to network with our panellists, Academy Fellows and other CATAC attendees at the end of this session.

Professor Dame Anne Johnson DBE PMedSci (chair)
Professor Anne Ridley FMedSci, University of Bristol
Professor Paul Stewart FMedSci, University of Leeds
Dr Michael Crichton, Heriot-Watt University

14:30 **Pre-Doctoral Plenary Competition**
Each competitor will have five minutes to present their research, followed by two minutes for questions

'Investigating intersectional inequalities in physical activity during the COVID-19 pandemic in UK adults' – Dr Lopa Banerjee, University of Sheffield and Sheffield Teaching Hospitals NHS Foundation Trust

'DEveloping Tests for Endometrial Cancer deTection (DETECT): a diagnostic accuracy study of urine and vaginal samples for the detection of endometrial cancer by cytology in women with postmenopausal bleeding' - Dr Eleanor Jones, University of Manchester and Manchester University NHS Foundation Trust

'Genetic liability to juvenile idiopathic arthritis is associated with cardiovascular phenotypes in early adulthood' - Dr Sarah LN Clarke, University of Bristol

'Is cam morphology a risk factor for osteoarthritis: findings from a causal analysis using Mendelian Randomisation?' - Dr Benjamin Faber, University of Bristol and North Bristol NHS Trust

'Elucidating the clinical spectrum and molecular basis of HYAL2 deficiency' - Dr James Fasham, University of Exeter and Royal Devon and Exeter NHS Foundation trust

'Iron: too much of a good thing?' - Dr Fergus Hamilton, University of Bristol and North Bristol NHS Trust

'Cervico-vaginal fluid protein signatures can accurately detect endometrial cancer: A proteomics based biomarker discovery study' - Dr Kelechi Njoku, University of Manchester

'Comprehensive steroid and global metabolome analysis by mass spectrometry and machine learning to understand metabolic risk in benign adrenal tumours with mild autonomous cortisol secretion' - Dr Alessandro Prete, University of Birmingham and University Hospital Birmingham NHS Foundation Trust.

15:30 **Refreshment break**

15:45 **Prize giving**
Professor Dame Anne Johnson DBE PMedSci

16:00 **Keynote**
Professor Charlotte Summers, University Lecturer and Honorary Consultant in Critical Care Medicine, University of Cambridge

16:45 **Closing remarks**
Professor Jane Norman FMedSci

16.50 - 18:00 **Networking and drinks reception**

Post-doctoral plenary talks

Dr Michael Drozd

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Non-communicable disease, sociodemographic factors, and risk of death from infection: a prospective cohort study of 493,295 UK Biobank participants

Background
Non-communicable diseases (NCDs) have been highlighted as important risk factors for COVID-19 mortality. However, insufficient data exist on the wider context of infectious diseases in people with NCDs. We aimed to investigate the association between NCDs and the risk of death from any infection before the COVID-19 pandemic (up to Dec 31, 2019).

Methods
We used data from the UK Biobank cohort to explore factors associated with infection death (censored to Dec 31, 2019). We used Poisson regression models including NCDs (obesity, hypertension, chronic heart disease, chronic respiratory disease, diabetes, cancer, chronic liver disease, chronic kidney disease, previous stroke, other neurological disease, psychiatric disorder, and rheumatological disease), age, sex, ethnicity, smoking and socioeconomic deprivation. All analyses were repeated with non-infection-related death as an alternate outcome measure to establish differential associations of infection death and non-infection death. Associations are reported as incidence rate ratios (IRR) accompanied by 95% CIs.

Findings
493,295 individuals were included. During 5·3M person-years of follow-up (median 10·9 years), 27,729 deaths occurred, of which 1,385 (5%) were related to infection. Advancing age, male sex, smoking, socioeconomic deprivation, and all studied NCDs were independently associated with both infection and non-infection death. Compared with white ethnicity, a pooled ethnic minorities group was associated with a reduced risk of infection death (IRR 0·65, 95% CI 0·46-0·87) and non-infection death (0·80, 0·75-0·86). Stronger associations with infection death than with non-infection death were observed for advancing age (e.g. age 65 vs 45 years: 7·59, 95% CI 5·92–9·73, for infection death vs 5·21, 4·97–5·48, for non- infection death), as was smoking, socioeconomic deprivation, class 3 obesity, hypertension, respiratory disease, chronic kidney disease and rheumatological disease. Accrual of multimorbidity was also more strongly associated with risk of infection death (five or more comorbidities vs none: 9·53, 6·97–13·03) than of non-infection death (5·26, 4·84–5·72).

Interpretation
Several NCDs are associated with an increased risk of infection death, suggesting that some of the reported associations with COVID-19 mortality might be non-specific. Only a subset of NCDs, together with the accrual of multimorbidity, advancing age, smoking, and socioeconomic deprivation, were associated with a greater IRR for infection death than for other causes of death. Further research is needed to define why these risk factors are more strongly associated with infection death, so that more effective preventive strategies can be targeted to high-risk groups.

Implications

People with some NCDs (together with multimorbidity, smoking, and socioeconomic deprivation) are at particularly increased risk of infection death, and strategies to discern and address the underlying mechanisms should be developed. Ongoing work addressing NCDs and sociodemographic factors as risk factors for fatal COVID-19 should also consider the wider context of how these factors are associated with death from any other infection and death unrelated to infection.

Dr Siying Lin

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Evidence that the Ser192Tyr/Arg402Gln in *cis* tyrosinase gene haplotype is a disease-causing allele in oculocutaneous albinism type 1B (OCA1B)

Background

Oculocutaneous albinism type 1 (OCA1) is caused by pathogenic variants in the TYR (tyrosinase) gene encoding the critical and rate-limiting enzyme in melanin synthesis. It is the most common cause of OCA in Caucasians, accounting for ~50% of cases worldwide. The apparent 'missing heritability' in OCA is well-described, with ~25–30% of clinically diagnosed individuals lacking two clearly pathogenic gene variants. Studies have suggested that the TYR p.(Ser192Tyr) and p.(Arg402Gln) variants, previously described as non-pathogenic polymorphisms due to their high frequency in the general population, may account for some of this missing heritability, although this has been heavily debated and not conclusively proven.

Methods

We performed empowered genetic studies, including exome sequencing and cosegregation analysis, in an extensive multigenerational Amish family with multiple affected individuals all exhibiting nystagmus and variable levels of hair and skin hypopigmentation, consistent with a diagnosis of OCA.

Functional tyrosinase activity studies were designed to study and quantify the effects of the TYR p.(Ser192Tyr) and p.(Arg402Gln) variants both independently and in combination compared to wild-type tyrosinase enzyme.

A retrospective analysis of genotyped UK-based albinism cohorts was also performed alongside a literature review evaluating the reported prevalence of the TYR p.(Ser192Tyr)/p.(Arg402Gln) haplotype in additional published OCA cohorts.

Findings

Clinical and genomic studies in four extended interconnecting Old Order Amish families identified previously described TYR variants p.(Ser192Tyr) and p.(Arg402Gln), as well as a likely pathogenic missense variant p.(Met252Arg), segregating with disease in multiple affected individuals with OCA. The TYR p.(Ser192Tyr)/p.(Arg402Gln) variants were linked in *cis*, and inherited in a compound heterozygous fashion with p.(Met252Arg) in all affected individuals with hypomorphic albinism (OCA1B); a single affected individual with complete albinism was instead homozygous for the p.(Met252Arg) variant.

Functional tyrosinase enzyme assays demonstrated a statistically significant cumulative effect of both p.(Ser192Tyr) and p.(Arg402Gln) variants on tyrosinase activity. Interrogation of in-house and published albinism clinical cohorts demonstrated an enrichment of the TYR p.(Ser192Tyr)/p.(Arg402Gln) haplotype in OCA cohorts with missing heritability (i.e. individuals in whom only a single pathogenic TYR variant has been identified) (50.7%) compared to molecularly diagnosed OCA cohorts (2.0%), and a control cohort of individuals with no OCA diagnoses (16.9%).

Interpretation

Together, our studies define the genotype, biochemical and phenotype correlation of the p.(Met252Arg) and p.(Ser192Tyr)/p.(Arg402Gln) TYR variants, and collectively demonstrate that the in *cis* p.(Ser192Tyr)/p.(Arg402Gln) allele is pathogenic, and contributes to an OCA1B diagnosis when inherited in trans with a second deleterious TYR variant. We

also show that homozygosity for the p.(Ser192Tyr)/p.(Arg402Gln) TYR haplotype results in a very mild, but fully penetrant, albinism phenotype. These data underscore the importance of including the TYR p.(Ser192Tyr)/p.(Arg402Gln) in *cis* haplotype as a pathogenic allele causative of OCA, which would likely increase molecular diagnoses in the missing heritability albinism cohort by 25–50%.

Implications

Our studies provide irrefutably strong evidence that the TYR p.(Ser192Tyr)/p.(Arg402Gln) haplotype is pathogenic, supporting a review of all previously undiagnosed OCA1B cases where these variants have been excluded, which could permit a significant uplift in confirmatory molecular diagnoses in a diagnostically challenging patient group.

In individuals heterozygous for this allele, alternative diagnoses such as syndromic albinism might be considered less likely, as they would be 'at least a carrier of a pathogenic OCA allele'. This avoids the need for further invasive investigations to confirm the clinical diagnosis or rule out syndromic forms of the disease or masquerading conditions, and permits re-examination of genomic data in a targeted fashion to search for further non-coding splice or structural variants in the TYR gene.

In individuals with a very mild albinism phenotype or isolated foveal hypoplasia, identification of this pathogenic allele in homozygous form may provide the molecular diagnosis, ending their diagnostic odyssey.

Dr Matthew Saunders

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Socioeconomic support to prevent tuberculosis among household contacts of people with tuberculosis in Peru: a community-randomised controlled trial

Background

Tuberculosis is a social as well as infectious disease: poorer, undernourished people living in densely populated areas are at higher risk of tuberculosis, and tuberculosis entrenches poverty by increasing costs, reducing income, and causing stigma and discrimination. The WHO recognises these inequalities and recommends socioeconomic interventions as part of a comprehensive strategy to end tuberculosis, which also emphasises focussing on prevention by scaling up provision of tuberculosis preventive treatment. We aimed to evaluate the impact of a socioeconomic intervention on tuberculosis diagnosis and preventive treatment completion among household contacts of people with tuberculosis in 32 impoverished urban communities in Peru.

Methods

Design. A community-randomised controlled trial with local and international ethical approval (https://www.isrctn.com/ISRCTN17820976).

Intervention. In 16 randomly selected supported communities, newly identified tuberculosis-affected households (people with tuberculosis and their household contacts of any age) were invited to consent to participate in a socioeconomic intervention, principally led by tuberculosis survivors. This consisted of integrated social support (household visits and tuberculosis clubs providing information, peer support, and assessment of tuberculosis risk for contacts); and economic support (monthly conditional cash transfers).

Analysis. Outcomes were compared with tuberculosis-affected households in 16 randomly selected comparison communities that received standard of care, with no additional intervention.

Findings

Recruitment. Between 2016-2018, 813 people with tuberculosis and their 2,600 contacts in supported communities, and 810 people with tuberculosis and their 2,099 contacts in comparison communities, were recruited and followed-up for 18 months, involving monitoring tuberculosis programme records and a final tuberculosis prevalence survey.

Tuberculosis diagnosis. Tuberculosis was diagnosed in 2.5% (65/2,579) of contacts in supported communities during 3,604 person-years of follow-up (incidence rate=1.4/100 person-years [95%CI=1.1-1.9]); and in 3.5% (72/2,086) of contacts in comparison communities during 2,872 person-years of follow-up (incidence rate=2.5/100 person-years [95%CI=2.0-3.2]). Contacts in supported communities were at lower risk of tuberculosis diagnosis during follow-up than contacts in comparison communities (hazard ratio=0.58 [95%CI=0.40-0.85], p=0.005).

Tuberculosis preventive treatment. Contacts in supported communities were more likely to start (46% [1,191/2,600] versus 16% [340/2,099], odds ratio=4.3 [95%CI=2.8-6.6], p<0.0001) and complete (40% [1,029/2,600] versus 11% [221/2,099], odds ratio=5.3 [95%CI=3.4-8.3], p<0.0001) six months of preventive treatment with isoniazid than contacts in comparison communities.

Interpretation

In this community-randomised controlled trial, household contacts of people with tuberculosis in supported communities were approximately 40% less likely to be diagnosed with tuberculosis during follow-up compared with contacts in comparison communities, partly because they were approximately four times more likely to complete tuberculosis preventive treatment. These findings provide rigorous evidence demonstrating the value of socioeconomic interventions for improving health among members of tuberculosis-affected households. Strengths include the robust community-randomised study design with prolonged follow-up including a prevalence survey to ascertain tuberculosis diagnosis. Limitations include that the study was limited to Peru, an upper-middle income country with low HIV prevalence.

Implications

This novel study is the first to demonstrate the effectiveness of socioeconomic interventions for preventing tuberculosis among household contacts of people with tuberculosis. The results provide timely and high-quality evidence to underpin the biosocial approach to ending tuberculosis outlined by the WHO, which calls for the integration of biomedical and socioeconomic interventions to address the poverty-related tuberculosis risk factors that drive global tuberculosis incidence. Our findings highlight the urgent need to scale-up and integrate socioeconomic interventions into practice in Peru and other settings globally with high rates of tuberculosis and poverty.

Next steps to build upon this research include: i) assessing the feasibility and cost-effectiveness of targeted strategies focussing socioeconomic interventions on households at highest risk of tuberculosis and/or socioeconomic adverse outcomes; ii) evaluating the impact of social versus economic versus integrated socioeconomic support; and iii) exploring how these interventions can be most effectively adapted and implemented in different settings.

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Harnessing liver-resident gamma delta T cells for immunotherapy of hepatocellular carcinoma

Background

More effective immunotherapeutic approaches are urgently needed for hepatocellular carcinoma (HCC). Gamma delta (γδ) T-cells are attractive candidates for cancer immunotherapy, due to their potent cytotoxicity, tissue localisation, and HLA-unrestricted tumour reactivity. We characterised liver and tumour infiltrating γδ T-cells in HCC, and explored whether modulating features of tissue-residency could provide a novel immunotherapeutic approach.

Methods

Lymphocytes isolated from paired blood, liver, and tumoural tissue from patients with HCC (n=29) in comparison to colorectal cancer liver metastases (n=30) were analysed by multiparameter flow cytometry. γδ T-cell counts were determined by immunostaining. Long-lived persistence of intrahepatic γδ T-cells was examined using donor and recipient HLA mismatched liver allografts (obtained 7-11 years post liver transplantation). Aminobisphosphonate (Zoledronic acid, ZOL) and IL-2 expanded blood Vδ2 T-cells, intrahepatic lymphocytes, and tumour-infiltrating lymphocytes, were co-cultured with human HCC cell-lines pre-treated with ZOL to promote tumour-cell phosphoantigen accumulation for Vδ2 T-cell receptor activation.

Findings

Higher intratumoural γδ T-cell counts were associated with smaller HCC size and greater 3-year patient survival ($p< 0.01$). γδ T-cells exhibited a tissue-resident memory T-cell (TRM) phenotype (CD69+CD49a+) in human liver and HCC, with superior anti-tumour cytokine production and long-lived persistence in the liver (>10 years); an attractive profile to recapitulate with immunotherapy. A subset of γδ T-cells, Vδ2 T-cells, were selectively depleted within HCC but displayed the highest γδ TRM phenotype. In vitro expansion of blood Vδ2 T-cells using clinically approved ZOL and IL-2 induced a de novo TRM phenotype with improved cytotoxicity. Furthermore, direct sensitisation of HCC cell-lines with ZOL enhanced the anti-tumour function of co-cultured expanded Vδ2 T-cells and Vδ2 TRM isolated from HCC livers and tumours, with a significant increase in tumour-cell lysis. Using an in vivo murine model, adoptive cell transfer of ZOL-expanded Vδ2 TRM combined with intratumoural ZOL delivery demonstrated the greatest HCC tumour regression.

Interpretation

Liver-resident γδ T-cells demonstrate beneficial and long-lived immunotherapeutic properties. Our findings indicate a novel immunotherapeutic strategy for HCC, combining the use of aminobisphosphonates to induce γδTRM for potential adoptive cell transfer, with intratumoural delivery to sensitise HCC for more efficient γδ T-cell based targeting.

Limitations and strengths: this research utilises human HCC cell-lines in vitro and within an in vivo murine model, which may not fully recapitulate the tumour microenvironment. Nevertheless, our data provide important insights into the beneficial properties of human liver γδ T-cells, and uncover a promising immunotherapeutic approach for HCC, with potential for translation to clinical practice.

Implications

Our results suggest that future trials could explore a dual immunotherapeutic strategy for HCC, combining de novo induced γδTRM capable of replenishing the depleted pool in HCC, with additional intratumoural delivery of ZOL for

enhanced tumour lysis.

In the broader context, the insights gained from this research may also be generalisable to other solid tumour types, as γδ TRM are likely to be long-lived in other human tissues and can infiltrate into other solid tumours. A combination approach of ZOL-expanded Vδ2 T-cells with direct intratumoural delivery of ZOL may enhance the anti-tumour responses of endogenous and adoptively transferred Vδ2 T-cells for other solid tumour types, potentially providing an alternative treatment option for patients resistant or intolerant to currently available immunotherapies. Furthermore, the HLA-unrestricted mechanism of γδ T-cell activation could enable the exciting prospect of a future 'off-the-shelf' pan-population cancer immunotherapy.

Pre-doctoral plenary talks

Dr Lopa Banerjee

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Investigating intersectional inequalities in physical activity during the COVID-19 pandemic in UK adults.

Background

Physical activity is important for maintaining physical and mental health; a lack of physical activity accounts for 1 in 6 deaths in the UK. The COVID-19 pandemic has impacted physical activity levels and exacerbated health inequalities, but how this impacted physical activity levels in different groups within society is not fully understood. This project aims to investigate the effects of the COVID-19 pandemic on physical activity levels, using an intersectional lens to better understand inequalities in different demographic groups, including socioeconomic factors, geography and specific characteristics.

Methods

An exploratory secondary data analysis using STATA and Excel of 20 self-reported online surveys completed by Savanta Comres for Sport England between 3/4/20 and 21/9/21 with approx. 2000 participants per survey and 36,000 people overall. Gender, age, ethnicity, socioeconomic status, urban/rural, region, access to a garden and occupation were investigated as independent variables (both additively and multiplicatively) and the dependent variable was the number of days exercised per week.

Findings

Overall, physical activity levels were highest in the 1st national lockdown and lowest during the 2nd and 3rd lockdowns. Being female, older, from a lower SES unemployed and not having garden access correlated to lower physical activity levels across the pandemic. Those living in rural areas were more active than urban areas throughout the pandemic, however, this reversed when society started to reopen. Several additive and multiplicative interactions were found, the most statistically significant of which included age, SES, urban vs rural and garden access.

Interpretation

The findings from this study affirmed existing inequalities (SES and occupation) and found that some inequalities narrowed over time (gender) and others widened (age and region). New inequalities were found, including living in an urban vs a rural area and having access to a garden. Findings on ethnicity were inconclusive. Further research is required to investigate the causes of these correlations.

Dr Eleanor Jones

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DEveloping Tests for Endometrial Cancer deTection (DETECT): a diagnostic accuracy study of urine and vaginal samples for the detection of endometrial cancer by cytology in women with postmenopausal bleeding

Background
Postmenopausal bleeding (PMB) prompts urgent investigation with sequential invasive and costly tests that can be painful or distressing for women. A simple, non-invasive test to identify cancer and safely reassure the 95% of healthy women with PMB would revolutionise patient care. Our group previously showed proof-of-principle that endometrial cancer can be detected in urine and vaginal fluid. The aim of this study was to prospectively validate the diagnostic test accuracy of urine and vaginal cytology for endometrial cancer detection in women with PMB.

Methods
In this prospective, multicentre diagnostic accuracy study, consecutive eligible women provided a self-collected voided urine sample and a Delphi screener-collected vaginal sample before undergoing routine clinical investigations for PMB. Samples were assessed by two independent cytologists blinded to participant cancer status. Discrepancies were settled by consensus review. Results were compared to those from standard clinical diagnostics including transvaginal ultrasound scan, hysteroscopy, endometrial biopsy and hysterectomy histopathology. Ethics committee approval was granted by North West-Greater Manchester West Research Ethics Committee (reference 16/NW/0660).

Findings
Of 1864 participants, 115 (6.17%) had endometrial (n=99) or pelvic malignancies (cervix-7, ovary-3, leiomyosarcoma-2, bladder-1, colorectal-2, metastatic pancreatic-1). The sensitivity and specificity of combined urine and vaginal cytology for endometrial or any pelvic cancer detection were 80.8% (95%CI: 71.7-88.0%) and 92.6% (95%CI: 91.2-93.8%), and 80.0% (95%CI: 71.5-86.9%) and 92.6% (95%CI: 91.2-93.8%), respectively. The negative predictive value was 98.8% (95%CI: 98.2-99.3%) for endometrial cancer detection and 98.6% (95%CI: 97.9-99.1%) for any pelvic cancer detection. Vaginal cytology performed better for endometrial cancer detection than urine cytology, with a sensitivity and specificity of 79.8% (95%CI: 70.5-87.2%) and 94.4% (95%CI: 93.2-95.5%) versus 73.5% (95%CI: 63.6-81.9%) and 94.7% (95%CI: 93.5-95.7%). Of the 19 endometrial cancers missed by urogenital cytology, 2 (10.5%) had high-grade histology and 1 (5.3%) was ≥stage-II, meaning that cytology detected 95.8% of aggressive histology and 96.4% of locally advanced or metastatic cases.

Interpretation
This novel diagnostic test holds great promise. It is minimally-invasive, simple to take and cytology results come back quickly. It has high sensitivity for high-grade or advanced disease and there are opportunities for repeat testing in the event of ongoing symptoms. It could be of particular benefit to those who are elderly, frail or who cannot tolerate current invasive diagnostic tests. Studies exploring its clinical utility are needed to determine how best to incorporate it into clinical practice in order to minimise unnecessary invasive tests for women who do not have cancer.

Dr Sarah LN Clarke

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Genetic liability to juvenile idiopathic arthritis is associated with cardiovascular phenotypes in early adulthood

Background
Juvenile idiopathic arthritis (JIA) is the most common paediatric rheumatic disease, associated with considerable morbidity. Systemic inflammation is implicated in accelerated atherosclerosis. There is increasing concern about the cardiovascular health of JIA patients given their early age of onset of inflammation and we have previously reported robust genetic correlation between JIA and coronary artery disease. However, guidance is lacking regarding cardiovascular risk factor assessment and modification for JIA patients, in contrast to their adult counterparts. This study aims to identify adverse cardiovascular phenotypes in early life associated with JIA polygenic risk, in order to inform cardiovascular risk assessment in JIA.

Methods
JIA polygenic risk scores (PRSs) were derived for 2,815 Avon Longitudinal Study of Parents and Children (ALSPAC) participants and validated using a positive and negative control design. The association between JIA PRSs and cardiovascular phenotypes at age 24years was assessed using linear and logistic regression. Cardiovascular phenotypes included anthropometry, blood pressure (BP), inflammatory and metabolic markers, lipid profiles, early atherosclerosis measures and echocardiographic assessments of cardiac structure/function. For cardiovascular phenotypes with strong evidence of association, we undertook a longitudinal analysis from age 7years to identify when these associations manifest. Ethical approval was obtained from the ALSPAC Ethics and Law Committee.

Findings
To enable comparison, all associations between JIA PRS and cardiovascular phenotypes were standardised, such that beta coefficients represent the standard deviation increase in the outcome per standard deviation increase in JIA PRS. JIA PRS was associated with diastolic BP (β 0.062, 95% CI 0.026-0.099, P=0.001), blood insulin (β 0.053, 95% CI 0.013-0.093, P=0.009), insulin resistance index (β 0.057, 95% CI 0.016-0.097, P=0.006), log high sensitivity CRP (β 0.049, 95% CI 0.007-0.091, P=0.024), waist circumference (β 0.039, 95% CI 0.003-0.074, P=0.034), fat mass index (β 0.052, 95% CI 0.017-0.088, P=0.004) and body mass index (β 0.046, 95% CI 0.011-0.081, P=0.009) at age 24years. For anthropometric measures and diastolic BP, there was suggestive evidence of association with JIA PRS from age 7years which strengthened and increased in magnitude up to 24years. Our findings were consistent across multiple sensitivity analyses, including across varying PRS P value thresholds (P <0.01 to P <5x10-8).

Interpretation
JIA genetic liability is associated with adverse cardiovascular phenotypes in young adulthood, supporting the hypothesis of increased cardiovascular risk in JIA. Our findings suggest that cardiovascular risk is a core feature of JIA, rather than secondary to the disease outcome/treatment, therefore JIA patients warrant screening for cardiovascular risk. The major strengths of this study are the rigorous cohort data used, and *a priori* decisions regarding PRS construction and validation. By examining JIA genetic liability rather than JIA diagnosis, we were able to investigate cardiovascular health in a large, sub-clinical sample without relying on clinical thresholds for diagnosis.

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Is cam morphology a risk factor for osteoarthritis: findings from a causal analysis using Mendelian Randomisation?

Background

Cam morphology, a bulging of the anterolateral aspect of the femoral head, has been associated with hip osteoarthritis (HOA) in observational studies. Cam morphology is postulated to cause HOA through impingement of the femoral head on the acetabulum. In response, surgical procedures to remove cam lesions have been developed with the aim of preventing HOA. Unfortunately, the studies which provide the evidence of this association are liable to unmeasured confounding and reverse causation. To overcome this, we aimed to use bidirectional Mendelian randomisation (MR) to establish whether a causal relationship exists between cam morphology and HOA.

Methods

Alpha angle (AA), a measure of cam morphology, was quantified on hip dual-energy X-ray absorptiometry (DXA) scans in UK Biobank (UKB), using a novel automated approach. We performed genome-wide association study (GWAS) of AA in UKB with replication in the Rotterdam Study, and used HOA summary statistics from the Genetics of Osteoarthritis consortium. Genetic instruments of AA and HOA were identified by linkage disequilibrium clumping of their GWAS summary result statistics (p-value $<5x10^{-8}$ & r^2 <0.001). Bidirectional two-sample MR was conducted using the inverse-variance weighted (IVW) method. Sensitivity analyses were done using MR Egger and weighted median (WM) approaches.

Findings

GWAS of AA (mean AA 47.8°, range 32-115, n=38,173) identified 6 independent loci associated with AA, namely *TGFA, TNFAIP8, TIAM2, LMX1B, SOX5 and UQCC1*. The *TNFAIP8* association signal colocalised with eQTL expression in degraded human cartilage. The GWAS of HOA (cases= 46,704; controls= 574,765; n= 621,469) revealed 72 independent genetic loci. The 6 and 72 genetic instruments of AA and HOA respectively were used in bidirectional MR. The results showed no causal effect of AA on HOA (IVW OR 1.87 [95% CI 0.94-3.72], P 0.07; MR Egger OR 0.01 [0.00-4.02], P 0.21, WM OR 1.27 [0.93-1.74], P 0.14). Rather the reverse was seen, with a genetic predisposition to HOA increasing AA (IVW β 0.07 [95% CI 0.04-0.11], P $3.08x10^{-06}$; MR Egger β 0.16 [0.06-0.26], P $2.70x10^{-03}$; WM β 0.05 [0.02-0.09], P $3.03x10^{-03}$). F-statistics suggested no weak instrument bias (all >34).

Interpretation

GWAS of AA identified 6 independent loci with biologically plausible roles in hip shape development. Our analysis suggests that *TNFAIP8*, expressed only in degraded cartilage, contributes to the development of cam morphology following joint degeneration. Subsequent MR analyses confirmed that a genetic predisposition to HOA leads to cam morphology, whereas cam morphology does not appear to influence HOA risk. These results suggest that the associations seen between cam morphology and HOA in observational studies are likely due to reverse causation, and as such targeting cam morphology as a way to prevent HOA is unlikely to prove effective.

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Elucidating the clinical spectrum and molecular basis of HYAL2 deficiency

Background

Our previous studies defined biallelic variants in HYAL2 as a novel cause of syndromic orofacial clefting in two nuclear families, with Hyal2 knockout mice displaying similar phenotypes. HYAL2, encoding hyaluronidase 2, undergoes complex cotranslational modification before achieving its final topology as a mature cell surface glycoprotein. It is proposed to catabolize extracellular hyaluronan, a key constituent of the extracellular matrix that provides the basement membrane for epithelial tissue formation. Here we better define the phenotype of the HYAL2-related syndrome and the pathomolecular basis of disease.

Methods

Ten affected individuals were identified through international collaborations and GeneMatcher. Clinical and genomic investigations were undertaken with informed consent alongside functional and in silico modelling studies of nine novel putative pathogenic variants. HYAL2 variants were introduced into human HYAL2 complementary DNA to assess impact on HYAL2 levels after transient expression in Hyal2–/– mouse embryonic fibroblasts. To explore whether HYAL2 variants led to endoplasmic reticulum-mediated degradation and/or failure in C-terminal GPI anchor addition, we performed immunoblotting and immunofluorescence using transfected cells under permeabilized conditions (intracellular HYAL2) and non-permeabilized (cell-surface HYAL2) conditions and analysed HYAL2 released from the surface by phospholipase C.

Findings

Our studies confirmed a recognisable craniofacial phenotype in addition to identifying severe complicated myopia, cleft lip/palate, and congenital cardiac anomalies as the most consistent manifestations of the condition. While orofacial clefting was initially found to be common, our studies indicate this represents a more variable clinical outcome. We also describe the first individuals with loss of function variants in trans with pathogenic missense variants, who notably exhibited cardiac anomalies at the more severe end of the spectrum, suggesting a potential genotype-phenotype relationship. In silico modelling of pathogenic missense variants identified likely deleterious effects on protein folding. Consistent with this, our functional studies show that although some variants permit intracellular expression of mutant HYAL2, all impact HYAL2 levels at the cell surface, with reduced or absent levels of mature HYAL2 detected here, providing experimental evidence for the pathogenicity of missense alleles.

Interpretation

Our clinical and genetic studies establish HYAL2 variants as a cause of syndromic orofacial clefting and indicate an emerging genotype–phenotype relationship regarding protein functionality and cardiac/ocular phenotype severity, which may aid refinement of clinical screening and enable interpretation of missense alterations beyond variant of uncertain significance. Our molecular findings identify HYAL2 deficiency as the disease pathomechanism with decreased hyaluronan and/or chondroitin sulfate degradation likely underlying ocular and cardiac disease. Together these studies expand understanding of the clinical spectrum and pathomolecular basis of HYAL2 deficiency, enabling us to propose clinical management guidelines for patients affected by this complex multisystemic disorder.

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Iron: too much of a good thing?

Background

Iron is a critical element for all eukaryotic and most prokaryotic life, and iron deficiency remains a major clinical problem. However, multiple randomised trials performed in low income settings have suggested iron supplementation is associated with an increased risk of infection and infection related death. There remains limited data on whether iron supplementation is associated with an increased risk of infection outside these settings. As there are known genetic variants associated with iron status, Mendelian Randomisation (MR) may - under certain assumptions - allow us to unpick whether iron supplementation is likely to be harmful.

Methods

We performed an MR analysis estimating the causal association between iron status and sepsis. Exposures were extracted from a large meta-analysis of three genome wide association studies (GWAS) on iron status, and outcomes were extracted from UK Biobank and FinnGEN. 453,169 participants enrolled in UK Biobank, 356,000 participants enrolled in FinnGEN, and between 131,471 and 246,139 participants were enrolled across the three iron biomarker GWAS. Two sample MR was performed for each iron biomarker and the outcome; results were combined in inverse-variance-weighted meta-analysis. Non-linear associations and the effect of baseline haemoglobin and ferritin were tested.

Findings

In observational data, a U-shaped association between ferritin levels and the risk of sepsis was identified, although risk of sepsis started increasing well within the accepted normal range of ferritin (inflection point: 105ug/L in men, and 68ug/L in women). In contrast, in inverse variance weighted mendelian randomisation analyses using UK Biobank as an outcome, increasing TSAT was associated with increasing incidence of sepsis (OR 1.10 for each SD increase in TSAT; 95% CI 1.03 – 1.17) with similar results for serum iron (OR 1.07; 95% CI 0.98 – 1.16), and ferritin (OR 1.09; 95% CI 0.99 – 1.2), with the opposite result for TIBC (OR 0.92; 95% CI 0.86 – 0.99). Effect sizes were similar in the replication cohort (FinnGEN), and were robust to sensitivity analyses of mendelian randomisation. MR estimates were unaffected by baseline haemoglobin and baseline ferritin (where available).

Interpretation

Increasing iron levels are causally associated with increased rates of sepsis in two healthy adult volunteer cohorts, with the strongest associations for TIBC and TSAT, both of which are rapidly altered by supplemental iron. These findings have implications for iron supplementation programmes in adults, and suggest caution in iron supplementation in the non-anaemic population.

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Cervico-vaginal fluid protein signatures can accurately detect endometrial cancer: A proteomics based biomarker discovery study

Background

Endometrial cancer is the leading gynaecological malignancy in high-income countries and its incidence is rising. Early detection has the potential to improve outcomes as treatment can be provided when it is most likely to effect a cure. Current tests for endometrial cancer diagnosis are invasive with low patient acceptability. Novel approaches for the detection of endometrial cancer based on non-invasive sampling methodologies and measurement of specific biomarkers for stratification could transform patient care. The anatomical continuity between the uterine cavity and the lower genital tract presents a unique opportunity for the exploitation of uterine derived biomaterial for biomarker discovery.

Methods

In this study, we recruited women with abnormal uterine bleeding as well as those with known endometrial cancer and carried out mass spectrometric analysis of cervico-vaginal fluid samples using sequential window acquisition of all theoretical mass spectra (SWATH-MS). A bespoke spectral library comprising 19,394 peptides and 2,425 endometrial cancer-related proteins was developed and validated for use in relatively quantifying and characterising putative biomarker signatures. Then, in the subsequent prospective study, we analysed cervico-vaginal samples acquired from an independent cohort of endometrial cancer cases and controls. Digitised proteomic maps were derived for each sample and analysed using machine learning methods.

Findings

In total, 118 women participated in the prospective study, including 53 (44.9%) with endometrial cancer and 65 (55.1%) with no evidence of cancer. Their median age and BMI were 57 years (Interquartile range (IQR) 52, 67) and 28kg/m2 (IQR 24, 34), respectively, and they were mostly of White British ethnicity (86.4% White). Most of the women had low-grade (64.1% grade I/II), early-stage (77.4% FIGO stage I), endometrioid (79.2%) endometrial cancers. A total of 598 proteins were quantified across the samples, 122 of which were up-regulated in the cancers and 161 were downregulated. Principal component analysis and Hierarchical clustering showed excellent separation between the cancers and controls based on the top 10 discriminatory proteins. A five-marker protein signature discriminated endometrial cancer from controls with an AUC of 0.95, validated in a subset of study samples. The incorporation of clinical risk predictors marginally improved the diagnostic prediction of endometrial cancer.

Interpretation

In this study, we exploit the natural shed of endometrial tumour secretions to develop a novel approach to endometrial cancer detection. We show proof of principle that endometrial cancers secrete unique protein signatures that can be detected in the cervico-vaginal fluid. This forms the basis for a simple, minimally invasive endometrial cancer detection tool. The protein panels identified in this study showed good accuracy for the detection of endometrial cancer to warrant clinical translation and have mechanistic links with the malignant transformation process. Whilst these results are promising, their prospective validation in a larger independent cohort must now be prioritised

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Comprehensive steroid and global metabolome analysis by mass spectrometry and machine learning to understand metabolic risk in benign adrenal tumours with mild autonomous cortisol secretion

Background

Benign adrenal tumours are discovered on cross-sectional imaging in 3-10% of adults and can be non-functioning (NFAT) or associated with adrenal hormone excess. Analysing 1305 prospectively recruited patients with benign adrenal tumours, we recently demonstrated that 45% of patients had mild autonomous cortisol secretion (MACS), i.e. biochemical evidence of cortisol excess but lack of distinct signs of Cushing syndrome (CS). We found that MACS increases the prevalence and severity of type 2 diabetes and hypertension and primarily affects women (Ann Int Med. 2022 Doi:10.7326/M21-1737). Here we analysed the cohort's steroid metabolome and non-targeted global metabolome to reveal underlying metabolic processes.

Methods

We analysed 24-h urine samples from all 1305 patients (649 NFAT, 591 MACS, 65 CS) using a 17-steroid liquid chromatography-tandem mass spectrometry (LC-MS/MS) profiling assay. In addition, we performed untargeted serum metabolome analysis by mass spectrometry in a representative sub-cohort (104 NFAT, 140 MACS, 47 CS) employing two complementary LC-MS assays, HILIC and C18-lipidomics, investigating water-soluble and lipophilic metabolites, respectively. Urine steroid and serum global metabolome data were analysed by two supervised machine learning approaches, generalized matrix learning vector quantization (GMLVQ) and ordinal regression (OR). Metabolites identified as most relevant by both approaches underwent pathway enrichment analysis.

Findings

Urine steroid metabolome analysis revealed an increase in glucocorticoid metabolite excretion from NFAT over MACS to CS, whereas androgen metabolite excretion decreased. Similarly, increased glucocorticoid metabolites and decreased androgen metabolites were the major differentiators between MACS patients with and without type 2 diabetes and hypertension, respectively. Lipidome analysis by both GMLVQ and OR identified glycerophospholipids, lysoglycerophospholipids, triacylglycerides, ceramides, sphingolipids, and acylcarnitines as the most relevant metabolite classes exhibiting gradually progressive changes with increasing cortisol excess over the spectrum from NFAT to MACS and CS. Pathway enrichment analysis revealed a distinct pattern of changes in amino acid and pyrimidine metabolism with increasing cortisol excess. Patients with type 2 diabetes showed additional distinct changes in acylcarnitines, bioactive lipids, and triacylglycerides.

Interpretation

Our results reveal a gradual change in the lipidome towards lipotoxicity with increasing cortisol excess. The observed changes in the steroid metabolome reflect increasing autonomous glucocorticoid production resulting in downregulation of ACTH-mediated adrenal androgen production. Both patients with type 2 diabetes and hypertension had increased glucocorticoid output and more adverse changes in the lipidome, indicative of a causative contribution of cortisol excess to their higher cardiometabolic burden. We provide mechanistic insights into the metabolic consequences of cortisol excess. Observed changes may hold promise for risk stratification in MACS, a highly relevant and previously largely overlooked metabolic risk condition.

Poster competition

Group A: Applied health services; Epidemiology; Population health sciences

A1 | Dr Chloe Barr

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The performance of HE4 alone and in combination with CA125 for the detection of ovarian cancer in an enriched primary care population

Background

Ovarian cancer is the leading cause of mortality among the gynaecological malignancies and survival improves with early diagnosis, however, detection in primary care is challenging. Current investigations lack the accuracy required for early diagnosis. Human Epididymis 4 (HE4) is a promising diagnostic biomarker, but has predominantly been studied in secondary care. We aim to investigate the clinical utility of serum HE4, alone and in combination with Cancer Antigen 125 (CA125) in a symptomatic primary care population. for the detection of ovarian cancer in a symptomatic

Methods

GP requested CA125 serum samples sent to Manchester University NHS Foundation Trust between April 2018 and April 2019 were eligible for inclusion. Following routine testing for CA125, samples were tested for HE4 using chemiluminescent enzyme immunoassays. Due to a low incidence of ovarian cancer in primary care, the group was enriched with pre-surgical serum samples from 81 women with epithelial ovarian cancer. The Risk of Ovarian Malignancy Algorithm (ROMA) score was calculated using age ≥51 years as a surrogate for menopause. Age-adjusted HE4 thresholds were derived empirically. Conventional diagnostic accuracy metrics were calculated.

Findings

1229 patients were included; 82 had ovarian cancer. Overall, ROMA performed best [AUC of 0.96 (95%CI 0.94-0.98, p=< 0.001)]. In women under the age of 50, the combination of CA125 and HE4 where either marker was positive, was superior [sensitivity-100% (95%CI 81.5-100.0), specificity- 80.1% (95%CI 76.7-83.1)]. In women over the age of 50, ROMA had best performance [sensitivity- 84.4% (95%CI 73.1-92.2), specificity- 87.2% (95%CI 84.1-90)]. Age adjusted thresholds added little in women under 50, however, improved specificity in women over 50. ROMA demonstrated superior diagnostic performance for early-stage disease [AUC 0.91 (95%CI 0.86- 0.96), p<0.001].

Interpretation

HE4 and ROMA may improve the accuracy of ovarian cancer diagnostic pathways in primary care, particularly for women under the age of 50 years, in whom diagnosis is challenging. To our knowledge, this is the first study investigating the utility of HE4 in primary care. A major strength of this study is that our control group were the population of interest. Limitations include; small case numbers, particularly in the under 50 group, ROMA diagnostic accuracy will be impacted due estimates based on age rather than menopause status, and enrichment will have impacted sensitivities. Larger prospective studies are required.

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Finding the Right FIT: One or Two Faecal Immunochemical Tests?

Background
Symptomatic referrals for investigation of suspected colorectal cancer (CRC) yields cancer in only 3-5%. Symptoms remain a poor discriminator of serious pathology and referrals are increasing without more cancers being diagnosed. This translates to delays in diagnosis for those with serious pathology, whilst those without undergo costly investigation with associated morbidity. Faecal Immunochemical Testing (FIT) is increasingly being used in symptomatic patients to prioritise requirement and urgency of investigations. A recent meta-analysis reported the one test sensitivity for detecting CRC was 84.4%. We aimed to investigate whether sensitivity of a single test could be improved by adding a second test.

Methods
A prospective sequential cohort study of patients referred via 'urgent suspicion of cancer' (USOC) and urgent pathways to secondary care with lower gastrointestinal symptoms was conducted. From January 2019 to February 2020, in the Single FIT Cohort (SFC), upon receipt of referral from primary care one FIT was sent to patients who would then be investigated as per standard practice. Between March 2020 and June 2021, Double FIT Cohort (DFC), a second FIT was offered on completion of the first. FIT positivity was set at 10µg Hb/g in keeping with the literature.

Findings
In the SFC 2260 patients completed FIT and colorectal investigation, sensitivity for CRC was 84.1% with a number needed to investigate (NNI) of 155 when FIT was less than 10µg Hb/g. 2412 patients of 3167 in the DFC completed two FITs and colorectal investigation. There were fewer CRCs with a FIT less than 10µg Hb/g in the DFC than SFC ($p<0.05$). Four hundred and fourteen (17.2%) of the DFC had discordant FIT results, containing nine CRC's (12.3%) and 33 (20.8%) CRC's or advanced adenomas (ACRN). Sensitivity for CRC in the DFC was 97.3% with a NNI of 825 when FIT was less than 10µg Hb/g. For advanced adenomas the sensitivity in the SFC was 51.4%, significantly improving to 67.4% in the DFC. The use of a double FIT strategy reduces the false negative rate by 81%.

Interpretation
This study provides a large, real-world analysis of using a single or double FIT strategy in secondary care. The double FIT strategy results in a clinically important improvement in sensitivity for colorectal cancer and ACRN over a single test approach which can reduce endoscopy demand by 68.4% with a very low missed cancer rate. It has been practice changing for patients with symptoms suspicious of colorectal cancer and influenced guidelines in Scotland for the application of FIT into colorectal service pathways.

A3 | Mark Gormley

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Pleiotropic Effect of Genetically-predicted Sexual Behaviour on Oropharyngeal Cancer Risk

Background
Human papilloma virus infection is known to influence oropharyngeal cancer (OPC) risk, likely via sexual transmission. However, sexual behaviour has been correlated with other risk factors including smoking and alcohol, meaning independent effects are difficult to establish.

We aimed to evaluate the causal effect of sexual behaviour, including age at first sex (AFS) and number of sexual partners (NSP), on the risk of OPC using Mendelian randomization (MR). MR is an approach to causal analysis which attempts to overcome shortcomings of conventional observational studies by using genetic variants which are known to be reliably associated with modifiable risk factors.

Methods
Genetic variants robustly associated with AFS and NSP were used to perform both univariable and multivariable MR analyses with summary data on (2,641 OPC cases and 6,585 controls), obtained from the largest available genome-wide association studies (GWAS). Given the potential for genetic pleiotropy, we performed a number of sensitivity analyses to account for horizontal pleiotropy including; MR of sexual behaviours on positive (cervical cancer/seropositivity for Chlamydia trachomatis) and negative control outcomes (lung/oral cancer), Causal Analysis Using Summary Effect estimates (CAUSE) and multivariable MR analysis to account for the effects of smoking, alcohol, risk tolerance and educational attainment.

Findings
In univariable MR, we found evidence supportive of an effect of both later AFS (IVW OR= 0.4, 95%CI (0.3, 0.7), per standard deviation (SD), p=<0.001) and increasing NSP (IVW OR= 2.2, 95%CI (1.3, 3.8) per SD, p=<0.001) on OPC risk. However, negative control analysis suggested potential violation of the core MR assumptions and subsequent CAUSE analysis implicated pleiotropy of the genetic instruments used to proxy sexual behaviours. Finally, there was some attenuation of the univariable MR results in the multivariable models (AFS IVW OR= 0.7, 95%CI (0.4, 1.2), p= 0.21; NSP IVW OR= 0.9, 95%CI (0.5 1.7), p=0.76).

Interpretation
A comprehensive series of MR analyses were employed in this study in an attempt to overcome the drawbacks of conventional epidemiological studies. However, MR makes various assumptions which if violated may generate spurious results. We initially observed an association between genetically predicted AFS and NSP and risk of OPC using univariable MR. Despite using genetic variants strongly related sexual behaviour traits in large-scale replicated GWAS, we found evidence for correlated pleiotropy (when variants affect multiple traits through a heritable shared factor). This emphasises a need for multivariable approaches and the triangulation of evidence when performing MR of complex behavioural traits.

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A randomised, controlled feasibility trial of problem-solving therapy for pregnant women experiencing depressive symptoms and intimate partner violence in rural Ethiopia

Background
There is an established bidirectional relationship between mental ill-health and intimate partner violence (IPV), including during and after pregnancy. The lifetime prevalence of IPV exposure among women in rural Ethiopia is as high as 72% and may be even higher during the perinatal period. Although a growing literature demonstrates the efficacy of brief psychological interventions in low and middle-income countries, few studies measure or examine the impact on pregnant women, or women experiencing IPV, especially in rural, low-income country settings. Pregnancy is the commonest time for Ethiopian women to access healthcare, making antenatal care (ANC) an important opportunity for intervention.

Methods
This randomised, controlled feasibility trial compared brief problem-solving therapy (PST) adapted for pregnant women experiencing IPV in rural Ethiopia (PST-IPV) with standard PST (not adapted for women experiencing IPV), and enhanced usual care (EUC). Amharic-speaking pregnant women attending ANC were screened for depressive symptoms and functional impact on the Patient Health Questionnaire, and past-year IPV exposure. Eligible women were invited to provide informed consent. Consenting women were randomised to four sessions of PST-IPV or PST delivered by trained ANC staff, or information about sources of support (EUC). Ethical approval came from King's College London (#HR-18/19-9230) and Addis Ababa University (#032/19/CDT).

Findings
Fifty-two participants were randomised to PST-IPV (n=25), standard PST (n=12), and EUC (n=15). Attending four sessions of PST-IPV, and implementing a randomised, controlled study design was acceptable to women and health workers, and feasible in rural Ethiopia. Evidence included a recruitment rate of 10 participants per week across two ANC services, 76% of participants randomised to PST-IPV completing four sessions, and 24% dropping out, usually due to moving away or postnatal confinement. Recommended adjustments for a future randomised controlled trial (RCT) included staggering recruitment in line with therapist availability (to address the unexpectedly high recruitment rate). Others included focusing therapist training on communication skills, automating randomisation decisions (to prevent protocol deviations), using a supervision cascade model (to address mental health specialists' centralisation in Addis Ababa), and conducting outcome assessments immediately post-participation and longer-term (to address difficulty with follow-up in the postpartum period).

Interpretation
A brief psychological intervention tailored for the needs of pregnant women experiencing depressive symptoms and IPV was acceptable to women and health workers, and feasible to implement in rural Ethiopia. Despite expectations of sensitivity, screening pregnant women for depressive symptoms and IPV in an ANC setting was acceptable and feasible, leading to a higher than expected recruitment rate. The randomised, controlled study design requires adjustment for a future, fully-powered RCT, including greater focus on training ANC staff in communication skills, prior to training them to deliver a simplified psychological intervention, tailored for this context.

A5 | Dr Joanna McLaughlin

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What effect have clinical commissioning group policies for thresholds of weight loss and body mass index had on access to knee replacement surgery in England?: An analysis from the National Joint Registry for England

Background
"The majority of Clinical Commissioning Groups (CCGs) in England have policies for thresholds of weight loss or body mass index for intervention prior to knee replacement surgery. Their stated intention is to improve clinical outcomes, reduce the need for surgery and trigger long-term lifestyle changes. There is significant geographical variation in intervention content, reflecting the current inadequate evidence-base for their effects. Potential unintended consequences include increasing health inequalities through differential impact on vulnerable sociodemographic groups' access to surgery.
Our aim was to assess the impact of these policies on access to knee replacement surgery in England."

Methods
Natural experimental study to compare the rate of primary knee replacement surgery and patient characteristics over time, between intervention policy clinical commissioning groups (CCGs) which introduced policies for patients with overweight or obesity between Jan 2013 and June 2018, and control group CCGs without a policy introduction. Data from the National Joint Registry (NJR) for England for 481,555 patients who had primary knee replacement between Jan 2009 and Dec 2019 in England were analysed using interrupted time series and difference-in differences analyses. Control and intervention CCGs were randomly matched. CCG level data were pooled relative to the policy introduction date.

Findings
Rate of primary knee replacement surgery per 100,000 population aged 40+ increased over time in all CCGs before policy introduction. While rates continued to increase in control regions, a sustained fall was observed in intervention regions after policy introduction (trend change -0.98 operations per quarter per 100,000 aged 40+ years, 95% confidence interval -1.156 to -0.803, P<0.001). For illustration, after 3 years at this trend, there are 11.8 fewer operations per quarter (0.98 x (3x4)) representing a fall of 17.5% from the rate at the time of policy introduction (67.2). These figures are 19.6 and 29.2% at 5 years. Rates of surgery fell in all patient groups, including non-obese patients. The proportion of independently-funded operations and patients living in the most affluent areas increased after policy introduction. There was no dose-response effect with policy severity and study patient and public involvement indicated policy severity is important to acceptability to patients.

Interpretation
Weight loss and body mass index policy introduction was associated with concerning decreases in the rates of knee replacement surgery. This study's powerful quasi-experimental study design examining policies introduced over a wide timeframe, and control group comparison, strongly reduces the likelihood of this effect being due to secular trends. A limitation of this study was the lack of available outcome data for non-surgical patients. The rate decrease affected all patient groups, not just the obese patients policies were targeted at. Changes in patient demographics seen after policy introduction suggest these policies have increased health inequalities and need urgent reconsideration.

A6 | Dr Fiona McQuaid

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Uptake of infant and pre-school immunisations in Scotland and England during the COVID-19 pandemic: an observational study of routinely collected data

Background

Maintaining high levels of childhood immunisation is vital for protection against infectious disease. The COVID-19 pandemic and control measures such as national lockdowns threatened to disrupt routine immunisation programmes, and initial reports from the start of lockdown in the UK and worldwide suggested that uptake was at risk of falling. In Scotland and England, enhanced surveillance of national data for childhood immunisations was established to inform and rapidly assess immunisation trends. The aim of this study was to use these data to assess the impact of the pandemic on infant and preschool immunisation uptake rates.

Methods

An observational study using routinely collected data obtained from Public Health Scotland ("COVID19 wider impacts on the health care system" dashboard) and ImmForm (England). Vaccinations delivered at 5 different ages were evaluated; three doses of '6-in-1' (DTaP/IPV/Hib/HepB) and two doses of Measles, Mumps, Rubella vaccine (MMR). Uptake during the 2020 lockdown was compared to the previous year (2019) using binary logistic regression analysis. For Scotland, timely uptake (within four weeks of eligibility) was analysed along with geographical region and deprivation index. For Scotland and England, we assessed whether immunisations were up-to-date at 6 (6-in-1) or 16-18 months (MMR) of age.

Findings

In Scotland, we found that timely uptake of all five vaccines was significantly higher during lockdown compared to 2019. Differences ranged from 1.3% for first dose 6-in-1 vaccine (95.3 vs 94%, OR compared to 2019 1.28, 95% CI 1.18-1.39) to 14.3% for second MMR dose (66.1 vs 51.8 %, OR compared to 2019 1.8, 95% CI 1.74-1.87). Significant increases in uptake were seen across all deprivation levels though, for MMR, there was evidence of greater improvement for children living in the least deprived areas. In England, fewer children due to receive their immunisations during the lockdown period were up to date at 6 months (6-in-1) or 18 months (first dose MMR). The fall in percentage uptake ranged from 0.5% for first 6-in1 (95.8 vs 96.3%, OR compared to 2019 0.89, 95% CI 0.86-0.91) to 2.1% for third 6-in-1 (86.6 vs 88.7%, OR compared to 2019 0.82, 95% CI 0.81-0.83).

Interpretation

We observed that the COVID-19 lockdown in Scotland was associated with an increase in timely childhood immunisation, however in England uptake fell slightly. The use of routine data used in this study was a limitation as detailed information on potential confounders were unavailable and we could not exclude the possibility of seasonal trends in uptake. Reasons for the improved uptake in Scotland should be explored further and may include more positive attitudes, improved accessibility or the reduction in barriers to immunisation. Promoting immunisation uptake and addressing potential vaccine hesitancy is particularly important given the ongoing pandemic and COVID-19 vaccination campaigns.

A7 | Benjamin Perry

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The Psychosis Metabolic Risk Calculator (PsyMetRiC): Development, External Validation and Beyond

Background

People who have psychotic disorders like schizophrenia die on average 10-15 years sooner than the general population, predominantly due to a substantially higher prevalence of cardiometabolic disorders. Signs of cardiometabolic dysfunction are commonly detectable from psychosis onset in young adults. In the general population, risk prediction algorithms like QRISK3 are routinely used to help clinicians target interventions toward those at greatest cardiometabolic risk. However, existing tools substantially underpredict cardiometabolic risk in young people with psychosis. Therefore, we developed and externally validated a cardiometabolic risk prediction algorithm tailored for young people with psychosis, prioritising clinical usefulness and patient acceptability.

Methods

We developed the Psychosis Metabolic Risk Calculator (PsyMetRiC) to predict 6-year risk of incident metabolic syndrome in people aged 16–35y with psychosis, using forced-entry logistic regression. We developed a full-model (including age, sex, ethnicity, body-mass index, smoking status, prescription of a metabolically-active antipsychotic medication, HDL and triglyceride concentration) and a partial-model excluding biochemical results. PsyMetRiC was developed and externally validated using electronic health record data from three UK psychosis early intervention services. Algorithm performance was assessed primarily via discrimination (C-statistic) and calibration (calibration plots). We did a decision curve analysis, and developed an online data-visualisation app. We followed TRIPOD guidelines.

Findings

651 patients were included in the development samples, and 510 in the validation sample. Sociodemographic characteristics were similar between samples, and the mean outcome prevalence was 18.76% across all three samples. PsyMetRiC performed well at internal (full-model: C=0.80, 95% CI 0.74–0.86; partial-model: C=0.79, 0.73–0.84) and external validation (full-model: C=0.75, 0.69–0.80; and partial-model: C=0.74, 0.67–0.79). At external validation, calibration of the full-model was excellent, but there was evidence of slight miscalibration of the partial-model. At a cutoff score of 0.18, in the full-model PsyMetRiC improved net benefit by 7.95% (sensitivity 75%, 95% CI 66–82; specificity 74%, 71–78), equivalent to detecting an additional 47% of metabolic syndrome cases. The online data-visualisation app is available at https://psymetric.shinyapps.io/psymetric/.

Interpretation

We have developed an age-appropriate algorithm to predict the risk of incident metabolic syndrome, an early precursor of cardiometabolic morbidity and mortality, in young people with psychosis. PsyMetRiC has the potential to become a valuable resource for psychosis early intervention service clinicians and could enable personalised, informed healthcare decisions regarding the choice of antipsychotic medication and targeted lifestyle interventions. We are now externally validating PsyMetRiC in a larger UK sample, and internationally in multiple nations. We are also taking steps toward integrating the algorithm in a complex physical monitoring and management intervention package for young people with psychosis.

A8 | Dr Laura Randall

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Utilising metabolomics and machine learning to develop early prediction methods for lameness in dairy cattle

Background

Lameness (impaired mobility) is an intractable condition in dairy cattle with profound welfare, health and economic implications, making it a top priority for livestock disease control in the UK. Detection of lameness is currently only possible at an advanced stage of disease when pain causes cows to walk with an altered gait. An early, automated method of detection would underpin fundamental improvements in the treatment and prevention of lameness. This study aimed to combine untargeted metabolomics and machine learning to evaluate a novel approach to early prediction of lameness in dairy cows.

Methods

Study subjects were 70 lame and 70 non-lame first lactation dairy cows. Urine samples were collected within 3 weeks pre- and post-calving and at the time of lameness. Samples were snap frozen in liquid nitrogen for storage at -80OC. Milk samples were collected at the time of lameness as dried milk spots on Whatman FTA DMPK-A cards, stored at room temperature. Urine Samples were analysed using untargeted LC-MS (Q-Exactive Plus mass spectrometer equipped with Dionex U3000 UHPKC system) and milk samples analysis used an automated sampling system TriVersa NanoMate LESA®. Data were analysed using a suite of machine learning methods.

Findings

All datasets were balanced for lame and non-lame cows, such that there were urine samples from one cohort of 45 lame and 45 non-lame cows, one cohort of 15 lame and 15 non-lame cows and milk samples from 10 lame and 10 non-lame cows. For urine samples collected at the time of lameness, the best machine learning algorithm (Support Vector Machine) provided a prediction accuracy for lameness of 82%. For urine samples collected pre- and post-calving the best models (Random Forest) achieved accuracies of 73% and 75% respectively. For milk samples collected at the time of lameness, all machine learning algorithms were able to predict lameness with an accuracy of 95-100%. Model triangulation identified 10 mass ions in milk, selected by multiple machine learning methods and with a high discriminant ability for lameness, to carry forward to pathway analysis.

Interpretation

This study demonstrated that untargeted LC-MS can be used to predict lameness in dairy cows with a high degree of accuracy and from samples collected non-invasively (urine and milk). Furthermore, samples collected as early as 3 weeks prior to calving (all lameness events occurred after calving) could be used to predict lameness, suggesting that development of tools utilising these techniques could offer huge opportunities for the early identification of high-risk cows to be targeted with additional preventive interventions. Further external validation work of this study, conducted on one herd, will be conducted in future.

A9| Dr Clare Shere

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Is musculoskeletal hypermobility associated with adolescent idiopathic scoliosis? A cross-sectional study in the Avon Longitudinal Study of Parents and Children (ALSPAC)

Background

Adolescent idiopathic scoliosis (AIS) is most often diagnosed during puberty and patients are followed up until the spinal curvature stabilises or progresses enough to require intervention. The ability to understand causes and predict progression of scoliosis is likely to strongly influence this management pathway. One potential predictor of progression is musculoskeletal hypermobility, but a recent systematic review (In Press) has highlighted a lack of population-based studies, use of non-validated measures of hypermobility and lack of adjustment for important potential confounders such as BMI.

Methods

We utilised a population-based birth cohort (the Avon Longitudinal Study of Parents and Children, ALSPAC) to investigate the association between musculoskeletal hypermobility at aged 14 and scoliosis at aged 15 in 4225 individuals. Musculoskeletal hypermobility, the primary exposure, was measured using the Beighton score. Spinal curvature was identified using a validated DXA-based method. The primary outcome was the binary outcome of yes/no scoliosis. Logistic regression was used to assess the relationship between musculoskeletal hypermobility and scoliosis. We investigated the influence of BMI, as ongoing work by another group suggests a sex difference in the association between adiposity and scoliosis.

Findings

The prevalence of musculoskeletal hypermobility was 19.6%, and the prevalence of scoliosis was 5.0%. Both hypermobility and scoliosis were more common among females. Hypermobile individuals were 1.48x more likely to have scoliosis compared to those without musculoskeletal hypermobility (OR 1.48 (95% CI 1.08,2.03), p=0.02). The strength of association did not differ between males and females, although separate analysis for males and females was limited by lack of power (for males OR 1.23 (0.58, 2.62), p=0.60; for females OR 1.22 (0.85, 1.75), p=0.28). We stratified by BMI categories in males and females due to differing directions of association between BMI and hypermobility and BMI and scoliosis. This suggested that in females the relationship between hypermobility and scoliosis is strongest in underweight individuals, whereas in males is strongest in obese individuals.

Interpretation

These results indicate a positive association between musculoskeletal hypermobility and AIS, with BMI influencing this association differently between sexes. Even in this large cohort there is limited power, highlighting a need to combine datasets for future analyses. This would allow robust analysis of factors influencing the association between hypermobility and scoliosis, including BMI as we hypothesise. The next step is investigating the association between hypermobility and curve progression using scoliosis data at aged 17 and 24 in the ALSPAC cohort. Delineating this complex relationship could help to identify patients at greater risk of curve progression.

A10 | Dr Gemma Simons

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Wellbeing in doctors: the measure matters! Development of a Core Outcome Set for measuring wellbeing in doctors

Background
The importance of doctors' health and wellbeing to everyone using Health Care is highlighted by the levels of burnout reported in doctors around the world. A number of UK policy documents made recommendations for the wellbeing of doctors, but how those wellbeing interventions are evaluated needs to be defined. Core Outcome Sets are increasingly being used in medicine to prevent waste in research, by recommending the inclusion of a minimum set of valid, reliable and practical measures. An operational definition and Core Outcome Set for wellbeing in doctors is needed to meaningfully progress the work in this field.

Methods
A critical review was undertaken to create an operational definition of wellbeing, which informed a systematic review of how doctor wellbeing has been measured. The acceptability and design of a Core Outcome Set was evidenced through Patient and Public Involvement (PPI), expert surveys and regional, and national, cross-sectional Surveys and interviews among doctors. A Delphi Study of doctors, and national stakeholders was undertaken to agree a Core Outcome Set, following Core Outcome Measurement in Effectiveness Trials (COMET) guidelines.

Findings
Systematic review identified 218 studies where wellbeing was an explicit outcome. Fifty-seven unique outcomes were identified, and the Maslach Burnout Inventory (n=18) was the most commonly used measurement tool. Cross sectional surveys (n=405) and interviews (n=11) confirmed that doctors felt a salutogenic Core Outcome Set was appropriate. The Delphi study (n=56) led to an agreed minimum set of 7 outcomes: General wellbeing, Health, Personal safety, Job satisfaction, Life work balance, Morale and Good clinical practice.

Interpretation
Use of a Core Outcome Set for wellbeing, alongside reporting standards and open access publishing by researchers, will ensure that when doctors take time to complete wellbeing surveys, they are evidence-based and make the data collected comparable. This will provide evidence for the system level changes that could really improve doctor wellbeing.

A11 | Rebecca Smith

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A retrospective review of acute gastrointestinal bleeding admissions in the early COVID-19 pandemic

Background
All healthcare services have been disrupted as a result of the COVID19 pandemic. There is little in the literature to suggest how acute gastrointestinal bleeding (AGIB) services have been impacted, although their reliance on timely aerosol-generating procedures does create a clear theoretical barrier to maintaining service quality.
A key performance indicator of an endoscopy unit's quality relates to the delivery of endoscopy for AGIB within 24 hours in 75% of cases. Timely endoscopy is associated with improved mortality and re-bleed rates.

Methods
A retrospective review of a Trust's endoscopy database was conducted, including cases of AGIB that occurred between 01/01/2020-01/03/2021. 805 cases were identified, 596 were excluded due to cases being unrelated to AGIB. 209 cases were included, data was gathered on demographics, time-to-endoscopy, COVID status, endoscopic diagnosis, post-endoscopic management, re-bleeding and mortality.

Findings
209 endoscopies were conducted with a median time-to-endoscopy of 20 hours (IQR 8-39). There were 38 episodes of re-bleeding and 36 deaths. COVID19 positivity was associated with a significantly increased risk of death, with a Chi squared test (4.46 p=0.035), however not with re-bleeding (0.009 p=0.922). Of five patients with AGIB and COVID19, two died because of respiratory disease, two further required ITU support for a mean of 21 days. One experience re-bleeding requiring embolization and another suffered a gastric perforation following endotherapy. Reductions in the number of cases of AGIB coincide with national lockdowns and mimicked a national cohort of non-COVID hospitals admissions. The month of April 2020 had the lowest number of cases at only 6, a figure that is an outlier at >1.5 standard deviations from the mean for the total study period. However, endoscopy units were able to deliver endoscopy for AGIB faster in national lockdowns.

Interpretation
AGIB cases reduced significantly during national lockdowns, suggesting patients were not willing or able to present to secondary care services. The combination of COVID19 and AGIB confers a very poor prognosis. These data suggest that in pandemics many cases of AGIB are managed at home with or without endoscopy; the outcomes of those non-attenders are unknown. It may be important to investigate whether this has resulted in an excess death rate from AGIB.

A12 | Dr Michael Tonkins

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In adult patients hospitalised in England and Wales due to low falls, is major trauma centre care associated with better outcomes than trauma unit care?

Background
Disability and death due to falls is a growing problem worldwide which disproportionately affects older adults. Optimising the management of these patients carries significant implications for the health of individuals and the performance of healthcare systems. However, studies concerning the benefit of higher-level trauma centre care have not yielded consistent, high-quality evidence of the role of higher-level care in patients injured by low falls. This study will research the effectiveness of major trauma centre care in adult patients injured by low falls in England and Wales.

Methods
In this retrospective cohort study, data were obtained from the Trauma Audit and Research Network (TARN) on adult patients (age over 16 years) injured by falls from <2 metres between 2017-2019 in England and Wales. 30-day survival, length of hospital stay and discharge destination were compared between major trauma centres (MTCs) and trauma units or local emergency hospitals (TU/LEHs). Binary logistic regression and Cox Regression were used to control for casemix.

Findings
127,334 patients were included of which 35,175 (27.6%) attended an MTC. Unadjusted 30-day survival was lower in MTCs (OR 0.69, 95% CI 0.66-0.73). After adjusting for casemix, MTC care was not associated with improved survival (adjusted odds ratio [AOR] 0.91, 95% CI 0.87-0.96).

Transferred patients had a significant impact upon the results. After excluding transferred patients, the AOR for survival in MTCs was 1.056 (95% CI 1.001-1.113). In non-transferred patients, the association between improved survival and MTC care was greatest in patients who suffered major trauma (AOR 1.126, 95% CI 1.044-1.215) and was absent in patients aged >65 (AOR 1.038, 95% CI 0.982-1.097). MTC care was associated with longer length of hospital stay (adjusted hazard ratio 0.906, 95% CI 0.894-0.918) and higher odds of discharge home (AOR 1.066, 95% CI 1.033-1.101).

Interpretation
TU/LEH care is at least as effective as MTC care due to the facility for secondary transfer from TU/LEHs to MTCs. In patients who are not transferred, MTCs are associated with greater odds of 30-day survival in the whole cohort and in the most severely injured patients. There is no association between MTC care and improved 30-day survival in patients aged >65 years. Future research must determine the optimum way to identify patients in need of higher-level care, the components of care which improve patient outcomes, and develop patient-focused outcomes which reflect the characteristics and priorities of contemporary trauma patients.

A13 | Dr Oliver Van Hecke

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A mixed-methods study to co-produce "evidence-based infographics" to increase parents' understanding about antibiotic use and antibiotic resistance

Background
Public misconceptions about antibiotic use persist despite the efforts of costly antibiotic awareness campaigns. These campaigns have often followed a top-down approach and have not sought input from the public in their design or content. One such group where better engagement is needed are parents or carers of young children. Preschool children have the highest antibiotic prescribing rate in the UK.

We aimed to co-produce a series of evidenced-based infographics (EBIs) on antibiotic use for common infections in children and to evaluate their effectiveness at increasing parents' understanding of antibiotic use and antibiotic resistance.

Methods
Mixed-methods design with three phases.
Phase 1: identify and summarise scientific evidence about antibiotics for three common childhood infections (sore throat, acute cough and otitis media) [rapid literature review]
Phase 2: co-production of prototype EBIs for each infection group to test their face- and content validity [iterative qualitative focus groups with parents of young children and graphic designers]
Phase 3: assess the effect of EBIs on parents' understanding of antibiotic use for the three infection groups [before-after national representative online survey in the UK]

Ethical approval: Medical Sciences Interdivisional Research Ethics Committee (IDREC), University of Oxford (R62414/RE001)

Findings
We iteratively co-produced ten prototype EBIs. Parents found the evidence displayed in the EBIs novel and relevant to their families. Parents did not favour EBIs that were too medically focussed. The way the information was displayed influenced their understanding. Parents preferred one health message per EBI.
We included eight EBIs in a national survey of parents (n=998). The median score percentage correct at baseline was 16% (IQR 10%–29%). EBIs improved knowledge by more than a third across the board (34%, IQR 20-46% p<0.001). Respondents confirmed that EBIs were novel and potentially useful, corroborating our focus groups findings.

Interpretation
Graphically representing evidence succinctly has the potential to change parents' perceptions about antibiotics for common infections. This is key to engage parents with awareness campaigns.
We employed a bottom-up approach by incorporating parents' beliefs about antibiotics and co-produced materials whose content resonates with parents. We used an iterative, cross-discipline process to test their face- and content validity. An important limitation is that a respondent's indication of their future behaviour in response to information may differ from what they would do in real life. Future research will test EBIs in real-world settings to assess their reach as a potential public-facing intervention.

Group B: Cellular and molecular biology; Genetics

B1 | Mr Adam Chambers

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Novel NF-κB signalling clusters predict DFS and OS following neoadjuvant therapy in rectal cancer

Background

NF-κB signalling is a primary regulator of inflammation alongside co-factors such as proto-oncogene BCL-3. In colorectal cancers, BCL-3/NF-kB expression correlates with poorer patient survival. DNA damaging therapies are commonly utilised in the neo-adjuvant setting for locally advanced rectal cancer. We showed BCL-3 loss results in sensitivity to DNA damaging therapies in murine and in-vitro colorectal cancer models. Further, canonical NF-κB signalling is activated following DNA damage. Therefore, understanding how BCL-3 and NF-κB signalling alters therapy response is critical to determining how this pathway contributes to outcomes. Here, we analyse data from the Stratified in Colorectal Cancer (S:CORT) consortium.

Methods

Access was granted to S:CORT (SCORT:TR031, MRC and CRUK funded). RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumour samples and RNA profiling performed using the Affymetrix Almac Xcel Array. Expression of RELA, RELB, NF-kB1, NF-kB2 and BCL-3 were used as inputs for data analysis using R (version 4.1.2 "Bird Hippie") and packages; "survival" (v3.2-13); "factoextra" (v1.0.7). Differential gene expression analysis was performed using "limma" (v3.50.0). Oncological outcomes (disease free survival (DFS) and overall survival (OS)) were analysed according to NF-kB cluster and BCL-3 expression.

Findings

Unsupervised k-means clustering identified three NF-kB clusters defined by expression of NF-kB subunit genes; Canonical (CC: RELA and NF-kB1 high), Non-canonical (NCC: RELB and NF-kB2 high) and atypical (AC: mixed expression between all genes) clusters. The CC was defined by significantly poorer DFS (p=0.0171) and OS (p=0.0327) compared to NCC and AC. Further, high BCL-3 expressing tumours within the CC predicted the poorest OS (Cox proportional hazards ratio 1.27 (SE 0.59, p=0.0308)) and DFS (COX proportional hazards ratio 2.02 (SE 0.84, p=0.0163)) compared to all other groups. We observed a number of genes were differentially expressed between NF-kB signalling clusters.

Interpretation

We have defined novel NF-kB signalling clusters that are predictive of survival in locally advanced rectal cancer. Determining how BCL-3/NF-kB signalling impacts survival of patients undergoing chemoradiotherapy for rectal cancer is critical. Defining this may facilitate treatment stratification and enable the development of novel therapeutics to enhance disease response for patients with locally advanced rectal cancer.

B2 | Dr Xinyi (Beibei) Du-Harpur

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Dissecting the cellular landscape of hidradenitis suppurativa

Background

Hidradenitis suppurativa (HS) is a chronic inflammatory dermatosis characterised by painful nodules, abscesses and sinus tracts, typically in flexural sites. It is common with an estimated prevalence of 2% and can be severely debilitating. Pathophysiology reflects the interplay of genetic susceptibility with environmental and lifestyle factors such as obesity and smoking. The inflammatory and immune landscape underlying HS remains poorly understood and treatment options are limited or only partially effective. My aim is to unravel the molecular signatures and pathways of this disease at single cell resolution using single-cell RNA-seq.

Methods

In order to better understand the cellular landscape of hidradenitis suppurativa, we have sequenced in excess of 30,000 cells from lesional skin of patients with hidradenitis suppurativa. This has revealed striking differences both in comparison to healthy skin and to other inflammatory skin diseases such as psoriasis and eczema. In ongoing work we are performing spatial transcriptomics with the 10X Visium platform and validating findings through immunostaining and in situ RNA FISH

Findings

We find over-representation of plasma cells in the cellular immune infiltrate in comparison with healthy and other inflammatory skin diseases. Further analysis of B-lineage HS cells shows increased expression of heavy chain genes and AICDA, which is critical for somatic hypermutation and class-switch recombination. Abnormal keratinisation is considered to be one of the first steps of HS pathophysiology, and my data shows a noticeable inflammatory profile in keeping with this. Further in silico pathway analyses exploring the relationships between cell types within HS implicate endothelial cells and fibroblasts as presenting key ligands that influence the gene expression profile found in HS keratinocytes.

Interpretation

Existing research into the pathophysiology of HS is noticeable in that the inflammatory signature implicates involvement of numerous cell types and pathways. Use of a variety of biologics targeting proposed molecules thus far have not shown a specific pathway being a sole driver of disease. The findings from my data so far suggest an interplay between endothelial cells, plasma cells and fibroblasts; this is supportive of a hypothesis that perhaps these cells form tertiary lymphoid tissues that uniquely perpetuate inflammation. Targeting this phenomenon, rather than specific pathways, may be a therapeutic approach to explore.

B3 | Alice Hughes

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A multi-ancestry genome-wide association study of umbilical cord insulin and C-peptide levels confirms expected relationships between glycaemic trait SNPs and fetal insulin and will form the basis of an important future bioresource

Background

Fetal insulin is a key regulator of fetal growth and the major contributor to high birthweight in pregnancies with maternal diabetes. There is evidence for genetic variation in regulating fetal insulin from studies of monogenic disease, and results of genome-wide association studies (GWAS) of birthweight show overlap with GWAS of common glycaemic traits. To investigate the genetic architecture of fetal insulin further, we performed a genome-wide association study (GWAS) of umbilical cord insulin and C-peptide measurements. We aimed to identify maternal and fetal genetic variants associated with fetal insulin levels and interrogate relationships with glycaemic traits and birthweight.

Methods

We included women and offspring from term pregnancies of diverse ancestry (six international studies with appropriate ethical approval and informed consent). We performed a multi-ancestry meta-analysis of umbilical cord insulin and C-peptide from term, singleton pregnancies without pre-existing diabetes. We tested whether genetic variants (single nucleotide polymorphisms, SNPs) known to be associated with fasting plasma glucose (FPG, n=90), insulin secretion (n=18), or both type 2 diabetes and birthweight (n=23; fetal effects only) showed more associations with umbilical cord insulin and C-peptide in the GWAS than expected by chance. We also tested whether the associations were in the expected directions.

Findings

We summarised association statistics with umbilical cord insulin and C-peptide for a total of 8,276,194 SNPs across 11,005 maternal genotypes and 8,175,230 SNPs across 10,630 fetal genotypes. No SNPs of genome-wide (P <5 x 10-8) significance were confidently associated with umbilical cord insulin and C-peptide. There was no enrichment of associations for SNPs associated with FPG, insulin secretion or type 2 diabetes and birthweight. However, pooled genetic effects had expected directional associations; e.g. pooled over 90 FPG SNPs, there was a 0.004 (95% CI -0.001-0.008) SD higher umbilical cord insulin/C-peptide per maternal FPG risk-raising allele and a 0.002 (95% CI -0.006-0.003) lower umbilical cord insulin/C-peptide per fetal FPG risk-raising allele. The pooled genetic estimate for the maternal insulin secretion SNPs showed the strongest evidence of association (0.01 (95% CI 0.002-0.03) SD higher umbilical cord insulin/C-peptide per maternal insulin secretion lowering allele.

Interpretation

This was the first GWAS of an umbilical cord biomarker and although the maximum number of available maternal and fetal genotypes was included, it was underpowered to detect important individual SNP associations with fetal insulin levels. However, expected directional effects for glycaemic trait SNPs were observed, supporting the hypothesis that these SNPs influence birth weight via an effect on fetal insulin-mediated growth. Additional samples will be added to develop this bioresource, enabling ongoing exploration of important research questions relating to fetal insulin.

B4 | Dr Rebecca Kaye

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Macular thickness varies with age-related macular degeneration genetic risk variants in the UK Biobank cohort

Background

Age-related macular degeneration (AMD) is a leading cause of vision loss in those aged 60 years and older. It is an irreversible destruction of the macula, which leads to the loss of sharp, fine-detail vision. Genetic risk is a key component of AMD susceptibility however, it is unknown if clinically normal individuals with AMD genetic risk alleles have previously unidentified macular changes.
The aim of this study was to evaluate the influence AMD risk single nucleotide polymorphisms (SNPs) have on outer retinal layer thickness at the macula in the UK Biobank population.

Methods

Unrelated UK Biobank participants of European ancestry with no history of ocular disease were included. All participants underwent genotyping and spectral-domain optical coherence tomography (SD-OCT) imaging. Those with poor image quality were excluded. All images underwent segmentation using the Topcon Advanced Boundary Segmentation (TABS) algorithm. To test the associations between selected AMD markers and retinal layer thickness we built linear models adjusted for age, sex, refraction, and smoking habits. We also computed polygenic risk scores (PRS) of AMD using alleles and effect and built linear models to assess PRS association with measurement of outer retinal layer thickness.

Findings

The final study sample included 32,113 UK Biobank participants. Mendelian randomisation modelling with AMD risk polymorphisms revealed a statistically significant decrease in thickness of the inner-segment outer segment (ISOS)-retinal pigment epithelium (RPE) thickness measurement, representing photoreceptor outer segments (POS), MR-Egger p=0.04. This same pattern was also seen when ISOS-RPE thickness was modelled using cumulative AMD risk in the form of a PRS (p=1.37x10-67). Increasing AMD-risk PRS was very significantly associated with a reduction in the thickness of layers that included the RPE. We saw complex additive effects of risk SNPs significantly regulating outer retinal layer thickness, most significantly rs10922109 within the CFH gene and rs6565597 at the NPLOC4-TSPAN10 locus, (beta= 0.25; p=0.0004). However, this association wasn't significant after Bonferroni correction (p=0.08). Very significant associations were also found between rs10922109 (p=1.47x10–49) and rs6565597 (p=1.00×10–38) and RPE-Bruch's Membrane (BM) average layer thickness.

Interpretation

The thickness of outer retinal layers is highly associated with the presence of risk AMD SNPs. Specifically, the ISOS-RPE measurement. Changes to ISOS-RPE thickness are seen in clinically normal individuals with AMD risk SNPs suggesting structural changes occur at the macula prior to the onset of disease symptoms or overt clinical signs. These results highlight the premorbid influence AMD genetic risk variants have on macular thickness and may provide mechanistic insight into the pathophysiology of this debilitating disease.

B5 | Annika Kröger

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Molecular Cartography of ‘Healthy’ and ‘Diseased’ Gingival Tissues

Background

Our group has in the past assessed the transcriptomes of ‘healthy’ and ‘diseased’ gingival biopsies to identify critical signatures of disease progression and different disease entities. However, these signatures represented only ‘mixed bags of cells’ and thus could not or only incompletely account for signatures of individual cell populations in specific layers of the gingival tissues.
We aim to identify the spatial transcriptomic signatures that (i) define the transition from ‘healthy’ to ‘diseased’ status, (ii) help explain the potential differences of grade B (‘moderate’) to grade C (‘fast progression’) periodontitis, and (iii) can be linked to specific bacterial-host interactions.

Methods

We have assessed a sample of 24 ‘healthy’ and paired ‘diseased’ gingival tissue samples from systemically healthy non-smokers using the Resolve Molecular Cartography technology, a proprietary serial FISH technology allowing for the simultaneous assessment of 100 transcripts in each tissue slide. We selected transcripts that allowed for the identification of critical cell types, invading periodontal bacteria and prominent transcripts informed by our previous whole-tissue approaches.

Findings

Specifically, 12 systemically healthy non-smokers contributed a ‘diseased’ gingival papilla (n=12; with bleeding-on-probing, probing depth ≥4mm, and clinical attachment loss ≥3mm), and a ‘healthy’ papilla (n=12; no bleeding-on-probing, probing depth ≤4mm, and clinical attachment loss ≤4mm). Data were assessed for specific differences between disease groups using mixed model regressions. Clusters of characteristic signatures were identified using mixture model-based clustering whilst accounting for covariates.
Our analyses revealed that specific spatial transcriptomic signatures exist that help differentiate 'healthy' and ‘diseased’ status, as well as different progression rates. These signatures can be contributed to defined cell populations and locations within the tissues, and co-locate in part with specific periodontal microbiota.

Interpretation

Our data on the molecular cartography of gingival transcriptomes in health and disease might help to improve early diagnostics of progressive disease, as well as inform targeted therapeutic approaches.

B6 | Dr Thomas Massey

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Exome sequencing of individuals with Huntington’s disease implicates FAN1 nuclease activity in slowing CAG expansion and disease onset

Background

Huntington’s disease (HD) is a progressive, untreatable neurodegenerative disorder that presents with a variable array of motor, psychiatric and cognitive symptoms. HD is caused by a CAG repeat expansion in the HTT gene over a threshold length of 35 repeats, with longer repeats associated with earlier onset disease. However, there is considerable variation in onset, some of which is heritable. Genome-wide association studies have identified a number of genes away from HTT that modify the onset of HD. Many of these genes encode DNA repair factors that might affect the stability of the CAG repeat in brain neurons.

Methods

We used exome sequencing of individuals with HD to look for rare protein coding variants in genes that modify HD onset. Identification of such variants could give insight into HD pathology. To maximise our power to identify rare variants we stratified 6086 HD patients from the European REGISTRY study by the difference between their actual age at onset and that predicted by CAG length alone and selected 250 at each extreme for sequencing. Rare variants were called, collapsed on genes and tested for association with HD onset. Some variants were then further analysed using in vitro and cellular assays.

Findings

We identified cis and trans modifiers of HD onset. Cis modifiers involved the DNA sequence just 3’ to the pathogenic CAG repeat: alteration of the canonical 5’-CAACAG-3’ to pure CAG was always associated with early onset HD ($p = 2.77 \times 10\text{-}7$) whereas extra CAA trinucleotides were associated with late onset HD ($p = 3.48 \times 10\text{-}6$). Significant trans modifiers included non-synonymous damaging variants in FAN1, a DNA repair nuclease. These were significantly skewed towards the early onset patient group (odds ratio = 3.43, 95% CI 1.66-7.09) and mapped to the DNA binding and nuclease domains of the protein. We purified some of these variants and found a significant correlation between greater nuclease activity and later HD onset ($p = 0.02$). Finally, we developed an induced pluripotent HD stem cell model of CAG expansion and showed that FAN1 nuclease activity slows expansions in a dose-dependent manner ($p = 0.003$).

Interpretation

Somatic expansion of the pathogenic HTT CAG repeat, most marked in the striatal neurons that degenerate first, might drive HD onset and progression. The disease-modifying variants identified here, both in DNA and modifier genes, provide mechanistic insight into somatic expansion and a framework for further experimental modelling. Heterozygous damaging coding variants in modifier genes can have a large effect on disease. Major strengths of this work are the extreme phenotype study design to maximise power and the use of human genetics to identify novel therapeutic targets. Limitations include the moderate sample size and the use of a single iPSC model.

B7 | Dr Lara Morley

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Piezo1 ion channels are important shear stress sensors in placental vasculature

Background

The mechanical force of blood flow is a fundamental determinant of vascular homeostasis. This frictional stimulation of cells, fluid shear stress (FSS), is increasingly recognised as being essential to placental development and function. Identifying specific mechanical sensors and the mechanisms governing how FSS force is converted into biochemical signals, is a fast-paced area of research. The mechanosensitive ion channel subunit, Piezo1, is an essential requirement for FSS sensing and normal maturation of murine embryonic vasculature. We tested the novel hypothesis that Piezo1 is important for human placental vascular adaptation, including release of the vasodilator, nitric oxide, and Notch signalling.

Methods

Patients delivering by elective caesarean section at term, with no maternal or fetal health concerns, were consented at Leeds Teaching Hospitals Trust. Primary fetoplacental endothelial cells (FpECs) were isolated from placental cotyledons using CD31-conjugated paramagnetic microbeads, and validated via responsiveness to VEGF, tube formation, viability assay, alignment to FSS and endothelial marker immunohistochemistry. Freshly isolated FpECs were used immediately for patch-clamp electrophysiology, or cultured to passage 5. RNA and protein expression were determined by QPCR and western blotting, respectively. Functional tests included short interfering RNA, application of Piezo1 modulators, ADAM10 secretase assay, response to FSS, Ca2+ measurements using the Flexststation.

Findings

RNA and protein expression of Piezo1 in FpECs was demonstrated, alongside patch-clamp recordings showing FSS-activated single channels characteristic of Piezo1. FpECs were highly responsive to FSS, showing alignment, upregulation of piezo1 RNA and protein, endothelial nitric oxide synthase (eNOS) phosphorylation, and ADAM10 activation (Notch signalling enzyme). The Piezo1 agonist, Yoda1, caused dose-dependent elevation of the intracellular Ca2+ concentration (EC50 5μM), and significantly increased eNOS phosphorylation (3 fold change). Yoda1 Ca2+ effect, and eNOS phosphorylation were suppressed following Piezo1 knockdown ($p<0.01$). Piezo1 depletion also resulted in loss of cell alignment to FSS ($p <0.05$) without losing cell viability.

We investigated Piezo1's involvement in additional downstream mechanisms important for vascular function – Notch signalling. Yoda1 evoked increased ADAM10 activation ($p=0.01$), and upregulated Notch genes, HEY1, HES1 and DLL4 ($p<0.05$). This was Piezo1 dependent, with blunted responses after Piezo1 depletion. All experiments were performed on ≥3 independent patient samples, and 3 replicates.

Interpretation

Piezo1 channels are present and functional in the fetoplacental endothelium, and disruption of Piezo1 prevents normal responses to FSS. As well as physiological stimulation, Piezo1 can be chemically modulated. We demonstrated that activating Piezo1 initiates eNOS phosphorylation, and subsequent production of nitric oxide is known to be the dominant placental vasodilator. We also demonstrated coupling of Piezo1 to downstream Notch signalling, which is important for angiogenesis and vascular remodelling. Understanding mechanisms through which FSS is sensed and how FpECs respond will provide novel insights into placental haemoregulation. This may have future therapeutic benefit for the treatment of placental vascular insufficiency.

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The effect of APOE genotype on astrocytic phenotypes in sporadic Alzheimer's disease (sAD)

Background

The *APOE E4* allele is the strongest genetic risk factor for sporadic Alzheimer's disease (sAD). *APOE44* homozygotes have a lifetime risk of almost 70% which is similar to genes in Mendelian diseases such as *BRCA1* in breast cancer. Despite its importance, the role of APOE remains enigmatic.

Astrocytes are the main source of CNS ApoE. Once considered mere 'scaffolding' it is now clear that astrocytes perform innumerable functions essential for CNS homeostasis. Perturbations in astrocyte physiology are implicated in several neurodegenerative diseases.

Our aim was to investigate how *APOE* genotypes affect astrocytic phenotypes thus contributing to the pathogenesis of sAD.

Methods

CRISPR/Cas9 gene editing was used to generate *APOE34* and *APOE44* isogenic lines from human embryonic stem cells (hESC) with an *APOE33* genotype. Successful editing resulted in a cytosine to thymine change, with a Cys112Arg substitution in the ApoE protein product, verified by Sanger sequencing of colonies derived from single cells. Quiescent astrocytes were derived from the isogenic *APOE33*, *APOE34* and *APOE44* lines using a novel protocol developed as part of the project. Astrocytes were then 'activated' with a cocktail of cytokines and experiments performed to identify key phenotypes in glutamate homeostasis, phagocytosis, cholesterol metabolism and inflammation.

Findings

When quiescent *APOE44* astrocytes were compared to *APOE33* cells:
1. reduced expression of APOE/ApoE: qPCR (*APOE33*=1.00, *APOE44*=0.25; $p=0.004$) and ELISA (*APOE33*=0.9ng/ug, *APOE44*=0.07ng/ug; $p<0.001$).
In 'activated' astrocytes:
2. impaired glutamate uptake: APOE33=3.04ng/ug, APOE44=1.36ng/ug; $p=0.048$. Further exploration of this phenotype confirmed differences in glutamate recptor expression (both gene and protein).
3. reduced phagocytosis: percentage phagocytosis was normalised to activity in quiescent *APOE33* cells; *APOE33*=201.3%, *APOE44*=27.2%; p=<0.001. *MERTK* gene expression which governs intermediate filament (GFAP and vimentin) production was reduced in *APOE44* cells ($p=0.033$). Protein expression of GFAP, but not vimentin, was significantly increased in *APOE44* cells (*APOE44*=0.93, *APOE33*=2.07; $p<0.001$).
4. perturbed cholesterol metabolism: *APOE44* astrocyte lysates and conditioned media contained significantly more cholesterol and cholesteryl esters. qPCR analysis of cholesterol metabolism genes found significant expression differences implicating impaired efflux and esterification mechanisms.
Data were obtained from 3 separate astrocytic differentiations and analysed using 2-way ANOVA plus appropriate post-hoc testing.

Interpretation

There are no efficacious disease modifying treatments for sAD. Anti-amyloid treatments have failed. Novel approaches are needed based on improved understanding of the disease's first cellular events.

I identified several *APOE44* dependent phenotypes converging on synaptic dysfunction; perturbed cholesterol metabolism which impairs the creation and repair of dendrites, decreased uptake of glutamate causing damaging excitotoxicity and reduced phagocytosis permitting accumulation of damaged synapses. Furthermore, we have recently acquired data that suggest these may be linked by a common 'druggable' pathway.

The main limitation was the use of 14-week astrocytes in experiments; prolonged differentiation produced more prototypic cells but cost restrictions precluded their use.

B9 | Dr Neil Ryan

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Distinguishing the molecular profile of endometrial cancer by spectroscopy: A Diagnostic Cross-Sectional Study

Background
Endometrial cancer (EC) is not a homogenous entity. The Cancer Genome Atlas (TCGA) demonstrated that there are in fact four distinct biological entitles, each with marked differences in prognosis. Furthermore, the different molecular groups are amenable to different treatments allowing personalised and effective treatment. However, molecular profiling is costly and timely which often leads to a delay in actionable results. Therefore, new technologies need to be explored that are rapid and less expensive. One potential technology is vibrational spectroscopy. The aim of this study was to examine the diagnostic characteristics of infrared spectroscopy in determining the molecular characteristics of EC.

Methods
Cases of EC were assigned to the four TCGA groups (path_POLE, Microsatellite high (MSI-H), copy number high and no-specific molecular profile) by recognised methods. Attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR) was used to collect ten spectra from different regions of each tissue section. Samples were divided into training (70%) and test (30%) datasets before further multivariate analysis to investigate whether spectra could be grouped in line with the established molecular phenotype. Principal component analysis linear discriminant analysis (PCA-LDA) was performed initially. This was followed by Principal component quadratic discriminant analysis (PCA-QDA) and support vector machine (SVM) analysis.

Findings
Overall, 314 ECs were included and two separate analyses were conducted; for the TCGA analysis 185 ECs were analysed.

PCA-QDA analysis of the 2800-3700 high spectral region performed best for the correct classification of EC into the four TCGA molecular groups with an overall accuracy of 99.1% and for the 900-1880 fingerprint region overall accuracy was 96.2%.

For MSS vs MSI-H, PCA-LDA and SMV algorithms applied to the high region both produced an accuracy, sensitivity and specificity of 78%, 100% and 33% respectively. Lynch syndrome associated endometrial cancer vs non-Lynch syndrome associated cancer (including somatic MSI-H) accuracy, sensitivity and specificity was 93% 100% and 70% respectively by SMV applied to the fingerprint region. In Lynch syndrome associated EC vs EC found to have MLH1 promotor region hypermethylation an accuracy, sensitivity and specificity of 83%, 100% and 75% respectively was returned by PCA-LDA analysis of the fingerprint region.

Interpretation
ATR-FTIR generated spectra enabled the accurate identification of EC into the four molecular groups identified by the TCGA analysis. In addition, it performed well in identifying Lynch syndrome associated EC even when compared head-to-head against EC with somatic MSI-H. These promising findings should be evaluated prospectively in larger studies.

Current delays in assigning EC to the TCGA molecular groups could be potentially overcome with the use of ART-FTIR. This could enable more timely selection of targeted treatments and improved clinical outcomes. In addition it could reduce the time and cost of Lynch syndrome screening in the EC population.

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Molecular dynamics analysis provides insights into ACTN1-related thrombocytopenia

Background
ACTN1-related thrombocytopenia (A-RT) is caused by heterozygous variants in ACTN1, encoding the cytoskeletal protein α-actinin 1. Variants are distributed throughout the protein with no clear genotype-phenotype correlation or common pathogenic mechanism. We hypothesised that pathogenic variants would be associated with an abnormal structural or molecular dynamic (MD) signature and aimed to demonstrate how different variants in the protein could cause the same clinical presentation.

Methods
A human α-actinin 1 structural homology model was generated and variants associated with A-RT introduced in hetero- and homodimeric states. MD simulations and principal component analyses (PCA) were performed using GROMACS software. The root-mean-square deviation of the protein backbone atoms was expressed in Ångstrom as deviation over time and deviation by residue. Global matrices were compiled to establish the most significant motions (principal components) for two variants (R320Q and R738W).

Findings
Initial analysis of the A-RT variant protein models did not identify a unifying structural abnormality. All A-RT variants produced local changes in intramolecular interactions, but with no gross distortion of the overall protein structure, including dimerization. PCA demonstrated no overlap in principal component (PC) 1 or PC 2 between the WT dimer protein and the R320Q or R738W variant models in hetero- or homodimeric state. As global matrices were used to generate the initial PCs, direct comparison between variants was possible. PCA demonstrated that the R320Q and R738W variant models were very similar to one another and easily distinguished from WT with regards to their motions.

Interpretation

These results suggest that both R320Q and R738W result in altered mechanical properties of α-actinin 1 in hetero- and homodimeric forms. This is a credible mechanism for thrombocytopenia as α-actinin 1 crosslinks critical structural elements in platelets, including the αIIbβ3 integrin, sub-integrin apparatus and the actin cytoskeleton. This is a preliminary finding based on analysis of only two A-RT variants. Further analysis of other variants including those not implicated in A-RT is required.

B11 | Dr Caroline Young

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The colorectal cancer (CRC)-associated faecal microbiome improves CRC screening accuracy: results from a diagnostic accuracy study of over 2000 NHS Bowel Cancer Screening Programme (NHSBCSP) card samples and a cross-sectional pilot study across three continents.

Background
Colorectal cancer (CRC) is the fourth cause of global cancer deaths and is increasing in non-Western cohorts. Research has indicated the potential for faecal microbiome analysis to improve CRC screening. However, this research predominantly analysed frozen stool (obviating clinical translation) and was largely conducted in Western cohorts.
We asked:
1) Could microbiome analysis be performed directly from NHS Bowel Cancer Screening Programme (NHSBCSP) card samples (guaiac faecal occult blood test (gFOBT) samples)? Would microbiome profiling improve CRC screening?
2) Could a similar methodology be used to profile non-Western cohorts? Does the CRC-associated microbiome of Western and non-Western cohorts differ?

Methods
Between 2016-2019 the NHSBCSP Southern Hub prospectively collected and anonymised processed gFOBT card screening samples with recorded colonoscopy outcomes. Samples were stored/transported at room temperature. DNA was extracted and V4 16SrRNA gene amplicon sequencing performed. Results were available for 2252 samples: blood-negative (n=491(22%)); CRC (n=430(19%)); adenoma (n=665(30%)); colonoscopy-normal (n=300(13%)); non-neoplastic (n=366(16%)). Analysis was performed using QIIME2, LEfSe and Random Forest.
The same methodology was performed on gFOBT card samples, collected with informed consent, from 10 treatment-naive CRC patients and 10 healthy volunteers, each from Argentina, Chile, India and Vietnam (total n=81).
Ethical approval: REC: 16/NE/0210; BCSPID_160; MREC17-077.

Findings
Random Forest models, based on relative abundance of genera, distinguished: CRC or neoplasm (CRC/adenoma) from blood-negative with AUC 0.86 (0.82-0.89) and AUC 0.78 (0.74-0.82); and CRC or neoplasm from colonoscopy-normal samples with AUC 0.79 (0.74-0.83) and AUC 0.73 (0.68-0.77). Models remained robust when restricted to 15 taxa, and external validation.

International samples were analysed collectively: CRC (n=41) and healthy volunteers (n=40). The microbiome remained stable despite international transport and storage of gFOBT samples at room temperature. Overall microbiome structure showed global differences associated with both continent and country. However, a collective CRC-associated microbiome was identified. This included Parvimonas, Peptostreptococcus, Fusobacterium, Alistipes, and Escherichia; taxa which were also CRC-enriched in the NHSBCSP cohort, and in external predominantly-Western CRC cohorts.
There were no adverse events.

Interpretation
1) Microbiome analysis of NHSBCSP samples shows potential to improve CRC screening. Microbiome-based models required as few as 15 taxa, raising the potential of an inexpensive targeted qPCR-based test. This could be integrated into the NHSBCSP at low-cost as an adjunct to existing screening, with the aim to reduce the number of unnecessary screening colonoscopies.
2) Identification of the same CRC-associated taxa in the international cohort reveals a universal CRC-faecal microbiome association, with the potential to develop a global microbiome screening test.
Strengths: NHSBCSP cohort; large numbers; validation; non-Western cohort. Limitations: limited NHSBCSP metadata; pilot international study.

B12 | Dr Julia Zöllner

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Rare variant contribution to cholestatic liver disease in a South Asian population in the United Kingdom

Background
Genes & health (G&H) is a population-based study of British Bangladeshi and Pakistani people (n=49,153) in the United Kingdom. G&H includes genetic data and linkage to electronic health records (EHR). Heterozygous ABCB4 (adenosine triphosphate-binding cassette, subfamily B, member 4) variants have been shown to contribute to the aetiopathogenesis of intrahepatic cholestasis of pregnancy (ICP) and low-phospholipid associated choleolithiasis whereas homozygous mutations in ABCB4 can cause a spectrum of disease from severe to mild progressive/benign familial intrahepatic cholestasis. This study aimed to assess the mutational burden of ABCB4 rare variants in this unique cohort.

Methods
We interrogated low/mid whole exome sequencing performed on 5236 volunteers reporting parental relatedness which had been processed using a standardised bioinformatic pipeline. Included were non-synonymous or loss of function (LoF) causing variants with a minor allele frequency (MAF) <5%. Variants were filtered and annotated if they met the following inclusion criteria: 1. associated with a phenotype 2. known in the literature 3. no recorded GnomAD allele frequency 4. predicted to be likely pathogenic (LP) based on 7 in-silico predictors. Variants associated with a phenotype in EHR or predicted to be likely pathogenic (LP) underwent protein structure and modelling analysis.

Findings
Out of 72 variants, 45 fulfilled the inclusion criteria and were heterozygous. Twenty-one were novel and unique to this cohort and not previously reported in the GnomAD database. Of those, 12 were considered LP and 2 pathogenic. Specifically, we identified 4 patients with ICP who carried each a missense variant of probable clinical relevance: G1254S, P1050S (novel), A833T (novel), and N510S. Based on in-silico prediction and the American College of Medical Genetics and Genomics criteria all 4 variants were considered as LP. We identified other variants that were associated with gallstone disease (n=7), cholangiocarcinoma (n=1), previously reported in the literature (n=9), and not previously reported (n=19). Four novel LoF variants were identified, three frameshift (associated with ICP: S99X; no associated

phenotype: F758X, and Lys30GlyfsTer7) and one introduction of premature stop codon (R595X; no phenotype). A total of 22 variants underwent protein modelling. The study identified

Interpretation

This study identified novel LP ABCB4 variants that appear to be unique to a South Asian population. We provide further insight into rare variants associated with ICP, cholangiocarcinoma, and gallstone disease. This study addresses the underrepresentation of diverse ancestry groups in genomic research.

Group C: Inflammation; Infection; Immunity

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Urine high risk human papillomavirus testing as an alternative to routine cervical screening strategy: the ACES Colposcopy Study

Background

Testing urine for high risk human papillomavirus (hr-HPV) may be an attractive option for non-attenders of routine cervical screening. The accuracy of urine hr-HPV testing varies with different collection protocols. We hypothesised that Colli-Pee has better sensitivity for CIN2+ detection than standard pot-collected urine through reliable first-void collection, standardisation of volume collected, and immediate preservative-fixation. The aim of the Alternative CErvical Screening (ACES) Colposcopy study was to compare the sensitivity of matched urine and cervical hr-HPV testing for CIN2+ detection using two urine collection devices.

Methods

Colposcopy attendees in Manchester (UK) with abnormal cervical screening results were randomised (1:1) to Colli-Pee® 10mls with preservative or standard pot for urine collection. Urine was self-collected and matched cervical samples taken immediately prior to colposcopy; hr-HPV testing used Roche Cobas 8800. Colposcopic opinion and/or histology informed clinical diagnosis. A power calculation indicated that 480 participants (with 120 CIN2+/group) would have 89.8% power to establish a sensitivity of urine for CIN2+ detection >80%.

Findings

324 participants were included in this interim analysis (Colli-Pee n=162, pot n=162; full data end March 2022). The groups were balanced in age (median 35.6 vs 35.8 years), ethnicity (77% vs 81% White) and referral screening results (n=74 vs n=73 high grade; n=68 vs n=71 low grade/borderline; and n=17 vs 17 persistent hr-HPV+/cytology-negative) in Colli-Pee and standard pot arms, respectively. Cervical hr-HPV was 96.6% sensitive (95%CI 92.2-98.9%) for CIN2+ detection (n=141/146). Urine hr-HPV sensitivity for CIN2+ was higher using Colli-pee (95.5%, 95%CI 87.5-99.1%, n=63/67) than when collected using the standard pot (75.0%, 95%CI 64.1-84.1%, n=60/80, p<0.001).

Interpretation

Hr-HPV tested Colli-Pee-collected urine shows similar sensitivity for CIN2+ detection compared to routine cervical screening. Further work in the general cervical screening population will establish its specificity and its potential to improve cervical screening uptake in current non-attenders.

C2 | Emily Milodowski

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Non-responsiveness to PD-1 blockade is associated with expansion and altered function of CD4+FoxP3+ tumour infiltrating Tregs

Background

Effective anti-tumour immune responses by cytotoxic tumour infiltrating lymphocytes (TILs) limit cancer development through recognition and destruction of cells with abnormal protein expression. During cancer progression, chronically stimulated TILs upregulate inhibitory immune-checkpoints and become functionally exhausted. Immunotherapies targeting immune-checkpoint PD-1 can re-invigorate responses of TILs by reversing PD-1-induced "off signals". However, PD-1 blockade monotherapy demonstrates limited efficacy in many cancers, and 60% of melanoma patients fail to respond. Multiple inhibitory immune-checkpoints are dynamically expressed in tumours. Improved understanding of how alternative checkpoints promote resistance to PD-1 blockade is essential for effectiveness of future therapies and identification of responding patient candidates.

Methods

8 week-old female BALB/c mice received subcutaneous injection of syngeneic murine renal carcinoma cells expressing novel antigen Haemagglutinin (RencaHA) cells. RencaHA tumour induction was confirmed on day 10 and mice were randomised into treatment groups: anti-PD-1 mAb (RMP1-14) or isotype control mAb (2A3); 250mg, i.p. q2d; 3 doses. Tumour volume was recorded every 48h using the following calculation: 0.5(length x width2). Tumour and lymph node tissue was harvested prior to phenotypic analysis via multicolour flow cytometry. Functional analysis of lymphocyte populations was performed by immunohistochemistry, proliferation suppression assays, and ELISpot. Experiments were conducted according to UK Home Office guidelines.

Findings

18 RencaHA tumour bearing mice received treatment with isotype or anti-PD-1 mAb. Response rate to PD-1 blockade was 33% (6/18), demonstrated by reduced growth rate (>1.8 standard deviations) compared to the control group. PD-1 blockade was associated with increased frequency of CD4+Treg TILs compared to control tumours ($p=0.0109$; T test). Elevated CD4+Treg frequency was also detectable in peripheral blood of non-responding mice compared to control ($p=0.000977$) or tumour-free mice ($p=0.0029$, one-way ANOVA and post-hoc Tukey). PD-1 blockade induced significant reduction in surface PD-1 expression ($p=0.00027$) and enhanced expression of costimulatory receptors including OX-40 ($p=0.0021$) within CD4+Treg populations. CD4+Treg TILs from RencaHA tumours were highly proliferative: 85.1% (mean) of CD4+Treg TILs were Ki67+, and functionally active: capable of suppressing antigen-specific CD8+ T cell proliferation and increased production of IL-10 after restimulation ex vivo ($p=0.0248$, paired T test). Immunohistochemistry revealed that CD4+Treg TILs occupied a spatially similar niche to CD8+ cytotoxic lymphocytes.

Interpretation

Failure of PD-1 blockade to control RencaHA tumours was associated with expansion of functional CD4+Treg populations in tumour and peripheral sites. These data demonstrate that, in additional to intended effects on cytotoxic TIL, CD4+Treg may respond to PD-1 blockade. The expansion of regulatory cells that can restrain anti-tumour responses by cytotoxic TILs represents a mechanism by which immune suppression may be maintained within the tumour microenvironment, despite PD-1 blockade, and reduce efficacy of such treatments. Selective targeting of intra-tumoural CD4+Tregs may improve patient responses to PD-1 blockade immunotherapy and warrants further investigation in the development of novel or combined immunotherapies.

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Interleukin 22 regulates neutrophil recruitment in ulcerative colitis and associates with treatment resistance to anti-cytokine therapies

Background

Interleukin (IL) 22 is a key cytokine involved in regulation of epithelial function and its role in IBD remains highly controversial. Some studies indicate a protective role in epithelial regeneration, whereas other studies imply a pro-inflammatory role. Insights are now needed to improve our understanding of the functional role of this important cytokine and how its expression might impact patients with ulcerative colitis (UC). In this study, we have probed the clinical and functional significance of IL-22 responsive transcriptional modules and causal networks in diseased tissue of over 500 UC patients and in preclinical models of colitis.

Methods

We mapped the transcriptional landscape of human colonic epithelial 3D mini-gut organoids in response to treatment with IL-22, and other pro-inflammatory cytokines. We tested the clinical significance of the IL-22 regulated transcriptome by probing whole colonic biopsies from 550 ulcerative colitis (UC) patients from the UNIFI clinical trial programme (patients with UC treated with ustekinumab, an anti-IL12p40 antibody) as well as reposited datasets with transcriptomic data of UC patients treated with anti-TNF therapy. The functional role of IL-22 regulated biological pathways were evaluated in pre-clinical models of UC.

Findings

IL-22 regulated pro-inflammatory biological pathways involved in microbial recognition, cancer and immune cell chemotaxis. IL-22 was an especially potent regulator of the CXC family chemokines which are all powerful neutrophil-selective chemokines. There was a positive correlation between the IL-22 transcriptional programme and the histological severity of inflammation ($r=0.49$, $p<0.0001$) and, in particular, neutrophil infiltration in lamina propria and the colonic epithelium of patients with UC. Patients with the greatest magnitude of enrichment for IL-22 responsive transcripts in whole colonic biopsies sampled immediately prior to ustekinumab initiation were less likely to achieve mucosal healing (8%) in comparison with patients with low enrichment scores (25%, $P=0.002$). A similar pattern was seen with anti-TNF treated patients. IL-22 mediated transcriptional regulation of CXC-family neutrophil-active chemokine expression was highly conserved across species and was dependent on JAK1/STAT3 signalling. In preclinical models of colitis, IL-22 induction of neutrophil-active chemokines was functionally and pathologically important.

Interpretation

This study provides new insights into cytokine mediated regulation of the intestinal epithelium and how this influences pathogenic pathways and patient outcomes in UC. We show that IL-22 is a functionally important regulator of neutrophil recruitment to the colon by controlling the expression of neutrophil-active CXC family chemokines. Augmented expression of IL-22 responsive transcripts and increased recruitment of colonic neutrophils was associated with treatment resistance to both ustekinumab and anti-TNF therapy. Refinement of this approach could herald a new paradigm for tailoring biologic therapy in UC.

C4 | Dr Sarah Prentice

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Can BCG protect neonates against diseases other than tuberculosis? Exploring the clinical and immunological non-specific effects of BCG through an investigator-blind randomised controlled trial in a setting with high infectious disease morbidity.

Background

Neonatal infections kill approximately 1 million children annually. Effective interventions to mitigate this unacceptable disease burden remain elusive.

Trials conducted in at-risk settings suggest that BCG vaccination reduces infant mortality more than would be expected by protection against tuberculosis. We hypothesised that these additional benefits result from protection against heterologous infections mediated by BCG-induced training of the innate immune system and designed a randomised controlled trial aiming to test this theory. No existing trial had purposely explored the impact of BCG on non-tuberculous infectious morbidity. Innate immune system training, formerly described in adults, had never previously been tested in neonates.

Methods

Healthy Ugandan neonates were recruited at birth following maternal written, informed consent, and randomised 1:1 to receive BCG (0.5ml SSI1331 intradermally) on day-of-life 1 or at age 6-weeks. Participants were actively followed-up to age 10-weeks for all-cause infectious disease outcomes, by clinicians blinded to intervention allocation. Blood samples taken at birth and four subsequent time-points contributed to immunological studies investigating:

- marks of chromatin accessibility at the promoter region of pro-inflammatory cytokines in myeloid cells
- cytokine production following ex-vivo whole blood stimulation with common neonatal pathogens
- the inflammatory:iron axis

LSHTM/MRC/UVRI Uganda ethics committees approved the study. Trial registration: ISCRTN#59683017.

Findings

560 Ugandan neonates were randomised to either receive BCG at birth (n=268 after withdrawals) or aged 6-weeks (n=269 after withdrawals). Risk of physician diagnosed, non-tuberculous infectious diseases was 29% lower during the first 6-weeks of life in those receiving BCG at birth compared to infants not yet BCG vaccinated (HR 0.71 95%CI (0.53-0.92) p=0.02). The reduction was particularly pronounced in low birth-weight infants (HR 0.10 (0.01-0.75), interaction p-value 0.04). Lower incidence was observed across diverse disease phenotypes and severities. After the delayed group received BCG there was no difference in all-cause infection incidence (HR 1.10 (0.87-1.40) p=0.62).

BCG at birth reduced the constitutive increase in histone trimethylation at pro-inflammatory cytokine promoters in myeloid cells over the first 6-weeks of life compared to BCG-naïve infants, significantly at TNF (H3K4me3 and H3K9me3 geometric mean fold-increase 3.1x lower (p=0·02) and 8·9x lower (p=0·005) respectively). Differences in down-stream innate immune effectors were not found.

Interpretation

BCG protects neonates in high morbidity settings against infectious diseases other than tuberculosis. This is likely mediated, in part, through training of the innate immune system via epigenetic modifications of myeloid cells. Further delineation of the underlying immunological mechanisms will be essential to allow the beneficial non-specific effects of BCG to be fully harnessed in the future. Optimisation of BCG delivery to ensure that all infants in high morbidity settings are vaccinated on the first day of life will have marked public health benefits beyond protection against tuberculosis and should be prioritised for public health programming.

C5 | Dr Michaela Reichmann

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Transcriptomic analysis of lymph node tuberculosis granulomas and a biomimetic model identifies sphingosine kinase 1 as a novel therapeutic target

Background

Currently a quarter of the world's population is infected with Mycobacterium tuberculosis (Mtb), killing 1.4 million people in 2019. Sarcoidosis is another granulomatous condition primarily affecting the lung and lymph nodes, sharing histological and clinical features with tuberculosis (TB) which can be indistinguishable. Immune-related phenomena occur in both diseases, suggesting shared immunological processes. We applied bioinformatic analysis to study treatment naïve TB and sarcoidosis lymph node samples, alongside control tissue. We aimed to identify pathways common to TB and sarcoidosis, and those unique to each condition, and investigate these in an advanced cellular model to identify novel host therapeutic targets.

Methods

Formalin-fixed paraffin-embedded mediastinal lymph node biopsies from seven TB, ten sarcoidosis and seven control patients were selected, none of whom had received antituberculous nor immunosuppressant treatment. Granulomas were excised using laser capture microdissection, with an equivalent area of control tissue excised. Total RNA was isolated, and sequenced by Ion Torrent Sequencing. In parallel, PBMCs from six healthy donors were infected overnight with Mtb strain H37Rv and incorporated into three cellular models of TB; 2-dimensional (2D), 3-dimensional (3D) microspheres with Alginate, and 3D microspheres with Collagen. Total RNA was extracted and sequenced by Illumina HiSeq. Consent was gained from all participants.

Findings

Bioinformatic analysis identified pathways shared by TB and sarcoidosis, namely extracellular matrix destruction and inflammatory response, most extensively regulated in TB. Disease-unique pathways were identified, including angiogenesis in TB and lysosomal activity in sarcoidosis. To find new targets, we used a systematic selection process to compare TB samples with infected 3D Collagen microspheres, identifying twelve potential targets including sphingosine kinase 1 (SphK1). SphK1 inhibition by PF-543 resulted in dose-dependent control of Mtb growth. Conversely, SphK1 activation by K6PC-5 increased Mtb growth. Furthermore, PF-543 reduced intracellular pH of infected monocytes, and suppressed inflammatory mediator secretion. Immunohistochemistry of TB lung biopsies confirmed SphK1 expression in granulomas. Experiments were performed on at least three occasions using PBMCs (three separate donors, triplicate conditions). Student's t test compared pairs, and ANOVA with Dunnett's correction used for multiple comparisons for groups of 3 or more. All results were significant (adjusted P value less than 0.05).

Interpretation

This work is the first published unbiased analysis of mediastinal lymph node TB, without the confounder of prior antibiotic or immunosuppressant treatment. SphK1 inhibition rapidly suppressed Mtb growth and normalised the intracellular pH of infected monocytes, suggesting the mechanism may increase phagolysosomal fusion, which Mtb inhibits as part of its intracellular survival strategy. By combining unbiased analysis of clinical samples with a biomimetic model, we have

established a translational pipeline to identify new therapeutic approaches. Enhancing host cell mycobacterial killing may shorten treatment duration and improve disease outcome. The limitation is we need to translate these findings to clinical trials.

C6 | Anika Singanayagam

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Characterising viable virus from air exhaled by pandemic H1N1 influenza-infected ferrets

Background

Each year seasonal flu affects 10-15% of the UK population and causes 12,000 deaths. In the rare but significant event that an influenza virus acquires capability to jump from animal-to-human host and efficiently transmit, a pandemic can result. For successful airborne transmission, virus must be exhaled in sufficient quantities and retain infectiousness in the air. There is a significant gap in knowledge about viral properties that enable survival of influenza viruses between hosts, due to a lack of experimental methods to isolate viable virus from air. Ferrets are considered the "gold standard" model for evaluating airborne transmissibility and pandemic potential.

Methods

We developed a novel apparatus to collect viable influenza virus from airborne droplets. The "transmission tunnel" contains 3 plates of susceptible cells onto which virus emitted from infected ferrets can deposit and be individually isolated. We used computational modelling to estimate droplet sizes collected. Using reverse genetics, we generated related influenza viruses that differ only by a single mutation that rendered one of the viruses less pH stable, and tested their ability to replicate in human airway cells. We inoculated ferrets with these viruses, sampled virus exhaled into the air and characterised it using deep sequencing and acid stability assays.

Findings

Ferrets infected with a pandemic H1N1 influenza exhaled a peak of infectious virus early after infection (day 1/2) but on subsequent days, when clinical symptoms became apparent and high levels of virus were still detectable in the nose, minimal infectious virus was collected from exhaled air. Virus engineered to be less pH stable replicated to high titre in the ferret nasal tract and was shed into nasal secretions. However, the pH-unstable virus displayed reduced survival in air, with very low amounts recovered from airborne droplets. Where virus could be recovered, it was identified genotypically as arising from minority viral populations in the nasal tract. These genotypic changes all resulted in reversion back to a stable viral phenotype. By nebulizing virus into the transmission tunnel, we could confirm our in vivo findings, demonstrating reduced survival of unstable virus in airborne droplets.

Interpretation

Virus stability is a key property facilitating airborne survival and between-host transmission, therefore an important parameter when risk assessing for pandemic potential of influenza viruses. Influenza-infected ferrets exhale contagious virus early after infection, before the onset of symptoms. The amount of virus in the ferret's nose is not a reliable indicator of the amount of viable virus in exhaled air. These findings, if demonstrated in influenza-infected humans, have implications for the ability of public health measures to detect and control influenza transmission.

C7 | Dr Chara Stavraka

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Predicting and preventing toxicities in patients with solid cancers treated with novel IgE class antibodies recognising tumour antigens.

Background

IgE antibodies (Ab) recognising tumour antigens are promising immunotherapeutic agents, with many patients expected to enrol in clinical studies. The anti-human folate receptor α (FRα) MOv18, the first-in-class IgE, is undergoing clinical testing. It has shown a favourable safety profile and signs of efficacy, with skin urticarial rash as the most common toxicity. Developing strategies to predict and prevent treatment-related toxicities would be key to maximising clinical benefit. We aimed to evaluate the propensity for toxicity of anti-tumour IgE antibodies in cancer patient groups likely to be offered this treatment.

Methods

We evaluated a panel of eight IgE antibodies with different antigen specificities engineered in human and rodent mammalian expression systems. We studied their propensity to trigger basophil activation using the Basophil Activation Test (BAT) in fresh whole blood from ovarian cancer (OC) patients and healthy volunteers (HV). Antibody glycosylation profiles were studied, and a glycan array was used to detect reactivity to glycans. The mechanism of the urticarial rash observed with MOv18-IgE was explored by immunohistochemistry (IHC), immunofluorescence (IF) and immuno-mass spectrometry (IMS), to evaluate whether FRα is expressed in the skin, and whether MOv18 recognises any other human antigen.

Findings

In a cohort of 25 OC patients and 15 HVs, blood basophil activation was not triggered by any of the IgE antibodies examined. The absence of FRα expression in human skin was confirmed by IHC. IF studies with fluorescently labelled MOv18 IgE on normal as well as urticarial skin from a patient treated with MOv18 IgE did not reveal any "off-target" binding of MOv18 IgE to human skin. This was supported by a proteome-wide IMS which confirmed that MOv18 did not recognise any antigens from a 20,000 human skin protein lysate. A 300 glycan array analysis also showed no specific binding.

Interpretation

In patient and HV cohorts, blood basophil activation was not observed with any of the IgE antibodies generated in different mammalian expression systems, adding further evidence that IgE therapeutics may not trigger systemic type I hypersensitivity reactions when introduced in the patient circulation. Our data suggest that the urticarial rash associated with MOv18 IgE immunotherapy is not caused by binding of MOv18 to the skin. Further work is in progress to predict potential toxicities and elucidate the mechanisms of IgE immunotherapy.

Poster competition

C8 | Paul Vulliamy

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The host-protective peptide Pentraxin 3 abrogates the toxic effects of extracellular histones on platelets: an in vitro modelling study

Background
Histones are damage-associated molecular patterns that are released into the extracellular space after immunogenic cell death. After major trauma, released histones exert direct cytotoxic effects on platelets which contributes to an acute coagulopathy. Pentraxin-3 (PTX3) is an endogenous protein released as part of acute phase responses that has histone-neutralising properties, but its effects in injured patients are unclear. In this study, we aimed firstly to determine how plasma histone and PTX3 levels relate to outcome in injured patients, and secondly to characterise the effect of PTX3 on histone-platelet interactions.

Methods
To quantify PTX3 and histones in trauma patients, we used an aptamer-based proteomic assay (SomaScan) in plasma samples taken within two hours of injury. Results are reported as relative fluorescence units. To examine the effects of PTX3 on histone-platelet interaction, platelets from healthy donors were incubated with histone H4 or vehicle in the presence or absence of PTX3. Platelet collagen receptors (GPIba and GPVI), activation status (p-selectin, CD62P) and histone-platelet binding were quantified with flow cytometry. Platelet ballooning and procoagulant transformation were measured using imaging flow cytometry. Results are reported as mean+/-95% confidence intervals from 6 independent experiments.

Findings
In trauma patients, plasma histones were significantly higher in non-survivors (7483+/-2311, n=23) than in survivors (3626+/-377, n=398; p<0.001). However, PTX3 levels were not significantly different (701+/-61 vs 628+/-25, p=0.16) and concentrations were an order of magnitude lower than concentrations of histones. In vitro, H4 induced a robust increase in platelet activation (CD62P MFI: 215+/-32 vs 11+/-5, p<0.001) and platelet ballooning (13+/-5% vs 1+/-1%, p<0.001) compared to vehicle, with parallel reductions in surface expression of GPVI (17+/-12 vs 97+/-19, p<0.001) and GPIba(60+/-30 vs 408+/-100, p<0.001). PTX3 prevented H4 binding to platelets (H4 MFI: 10+/-5 vs 162+/-48, p<0001) and almost entirely inhibited H4-induced platelet membrane disruption as quantified by ballooning morphology (3+/-1% vs 13+/-5%, p=0.002) and annexin V binding (18+/-9% vs 60+/-14%, p<0.001). Similarly, platelets exposed to H4 in the presence of PTX3 had significantly higher levels of GPVI (62+/-30 vs 17+/-12, p=0.009) and GPIba(255+/-57 vs 60+/-30, p<0.001) compared to H4 alone.

Interpretation
These results indicate that PTX3 protects platelets from histone-mediated membrane damage and collagen receptor downregulation. In trauma patients who do not survive their injuries, circulating histones are markedly elevated without a concomitant increase in PTX3 levels. Further study is warranted to evaluate the efficacy of PTX3 as a treatment for injury induced platelet dysfunction.

Group D: Neuroscience; Imaging; Technology

D1 | Dr Michael Ambler

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Synthetic torpor: taking the insult away from the injury in critical illness?

Background
Intensive care practice suffers a paradox: life-supporting interventions designed to increase delivery of oxygen and nutrients to vital organs frequently cause harm. Torpor is a hypothermic, hypometabolic, and bradycardic organ-protective state employed by mice in response to energy deficit. If mimicked in a clinical setting, it might allow patients to tolerate cardiac or respiratory failure, reducing the need for invasive interventions. Neurons in the preoptic area of the hypothalamus (POA) appear to trigger torpor in the mouse. We hypothesised that rats, which do not naturally enter torpor, can be induced into a similar state through activation of conserved neural circuits.

Methods
Designer receptors exclusively activated by designer drugs (DREADDs), such as the modified metabotropic acetylcholine (ACh) receptor hM3Dq, allow control of specific neurons in-vivo. hM3Dq is unresponsive to endogenous ACh but depolarises neurons in response to clozapine-N-oxide (CNO). We employed genetically engineered adenoviral vectors injected directly into the rat hypothalamus to express DREADDs in excitatory neurons of the preoptic area (POA). We then activated those POA neurons by intraperitoneal injection of CNO and recorded surface temperature and oxygen consumption. Next, under isoflurane anaesthesia and using a heat mat to maintain normothermia, we recorded heart rate while activating these same POA neurons.

Findings
Targeted activation of excitatory neurons in the POA of the rat induced a state of reduced body temperature (before versus after CNO, paired $t(4) = 8.40$, $p < 0.01$), reduced oxygen consumption (before versus after CNO, paired $t(2) = 6.15$, $p < 0.05$), and a sinus bradycardia that was independent of body temperature (repeated measures ANOVA CNO versus saline, interaction treatment by time, $p < 0.0001$). We term this state 'synthetic torpor'. CNO injection to control rats that underwent sham vector injection did not show any temperature or oxygen consumption response. Rats recovered from repeated bouts of synthetic torpor with no apparent effect on thermoregulation or behaviour. Hence, by activating neurons in the rat POA, we have recapitulated three cardinal features of torpor.

Interpretation
This work supports the hypothesis that conserved neural circuits exist in species that do not naturally enter torpor, and that exogenous activation of those circuits can induce a synthetic torpor-like state. Future work will focus on understanding the role of these circuits in species for which torpor is not an extant behaviour, will explore the circuitry and phenotypes of these neurons, test whether additional species are also able to enter synthetic torpor, and investigate the hypothesis that synthetic torpor allows animals to better tolerate injury to the heart or lungs.

D2 | Dr Haotian Gu

King's College London

Relationship of diastolic function and clinical outcomes to early systolic function: evidence for impaired early systolic function as the primary cardiac abnormality in HFpEF

Background

Approx. 50% patients with heart failure have preserved ejection fraction (HFpEF) and the prognosis is poor. The primary cardiac pathology is thought to be impaired diastolic relaxation of the left ventricle. However, we have previously shown that patients with hypertension who are predisposed to HFpEF have an impairment of early systolic function as measured by a novel imaging marker - first phase ejection fraction (EF1). EF1 is the ejection fraction up to the time of maximal ventricular contraction.
The objective was to examine the relative prognostic impact of EF1 compared to BNP and diastolic function measures in patients with HFpEF.

Methods

Consecutive patients presenting with suspected decompensated heart failure (HF) who underwent BNP testing and echocardiography and were diagnosed with HFpEF according to European Society of Cardiology guidelines at Guy's and St Thomas' Hospitals were included.
The primary endpoint was a combination of first re-hospitalization for HF or all-cause mortality.
The study was approved by the local research ethics committee and compiled with the Declaration of Helsinki.
Echocardiography images were analysed according to the recommendations of the American Society of Echocardiography. EF1 was measured as the percentage change in LV volume from end-diastole to the time to peak AV flow velocity.

Findings

In 177 patients [mean age: 75.8 (SD:12.9) years, 59.9% female] followed for a median of 19.3 months, 101 patients reached the primary endpoint (40 deaths and 61 hospitalisations). EF1 was negatively associated with N-terminal pro-brain natriuretic peptide (NT-proBNP) (B= -0.185, p=0.014).
ROC analysis showed that EF1 had the largest area under the curve (0.727) compared to other echocardiographic and biochemical predictors (p<0.001).
EF1 was the most powerful predictor of events. A optimal cut-off value of 19.4% gave hazard ratios (for EF1<19.4% compared to ≥19.4%) of 2.582 (95% CI: 1.708–3.905, p<0.001) unadjusted and 2.750 (95%CI:1.737–4.353, P<0.001) , adjusted for age, gender, other echocardiographic indices [including EF, E/e' ratio, end-diastolic volume, stroke volume and left atrial volume index (LAVi)] and NT-ProBNP. The c-statistic index for logistic model (including age, NT-proBNP, LV mass, LAVi, E/e' and presence of pulmonary hypertension) increased significantly by adding EF1 (0.650 to 0.747, p< 0.01).

Interpretation

EF1 was a much stronger predictor of clinical outcomes, than any biomarker described to date. This supports the view that systolic dysfunction is central to HF with both preserved and reduced EF and is an important determinant of prognosis in both conditions. HFpEF is a heterogenous condition associated with multiple comorbidities, our findings suggested that despite such comorbidities there may be considerable scope to reduce morbidity and mortality through focusing on systolic function. EF1 as a simple marker could be used to guide treatment through optimisation of loading conditions and improvement of early systolic function may improve outcomes in HFpEF.

D3 | Dr Robert McCutcheon

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Dopaminergic organisation of the striatum is linked to cortical activity and brain expression of genes associated with psychiatric illness

Background

The neuromodulator dopamine strongly influences the ability of neural circuits to control behaviour. Dopamine signalling is constrained to discrete tracts yet has brain wide effects on neural activity. The nature of this relationship between local dopamine signalling and brain wide neuronal activity is not clearly defined and has relevance for neuropsychiatric illnesses where abnormalities of cortical activity and dopamine signalling coexist.

Methods

We employed simultaneous PET-MRI to measure striatal D2/3 receptor availability using the radiotracer [11C]-(+)-PHNO, and cerebral blood flow using arterial spin labelling. 52 scans were obtained from 28 volunteers, with one scan following placebo administration and the other following dexamphetamine administration. We integrated high-dimensional data from PET and MRI in an unbiased manner with canonical correlation analysis to identify mappings between striatal dopamine and cortical activity. We next examined whether this mapping linked changes in striatal dopamine and cortical activity following amphetamine administration, how this mapping related to gene expression data, and whether genes implicated were overexpressed in psychiatric disorders

Findings

We identified a strong mode of covariation between striatal dopamine signalling and cortical blood flow (out of sample cross validation p<0.001), and demonstrated that spatial patterns of striatal dopamine signalling predict patterns of cortical blood flow (accuracy 81%, p<0.001). This mapping linked amphetamine induced changes in striatal dopamine receptor availability to changes in brain wide blood flow (p=0.04). Striatal gene expression patterns were associated with this mapping (p=0.04), and the implicated genes overlapped significantly with genes upregulated in schizophrenia, bipolar disorder and autism (p<0.001).

Interpretation

These results advance our knowledge of the relationship between cortical function and striatal dopamine, with relevance for understanding pathophysiology and treatment of diseases in which simultaneous aberrations of these systems exist.

D4 | Dr Christopher Osuafor

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Improving the visualisation of Lenticulostriate Arteries at 7 Tesla MRI using Contrast Enhancement

Background
Cerebral small vessel disease is a major cause of stroke and vascular dementia. Until recently, it has not been possible to visualise the small cerebral perforating arteries, like the lenticulostriate arteries (LSAs), non-invasively. This is now possible using the higher resolution at 7 tesla (7T) MRI. Contrast-enhanced magnetic resonance angiography (CE MRA) has been widely used for visualisation of larger extra-cerebral carotid and vertebral arteries however, Its utility for the visualisation of LSAs, is not well known. In this study, we compared CE MRA with non-contrast MRA at 7T to assess for an improvement in the visualisation of lenticulostriate arteries.

Methods
Ten patients with lacunar stroke due to cerebral small vessel disease (mean age 64±9.9 years) underwent whole-body human 7T MR system (7T Terra, Siemens Healthineers). Participants prospectively had Time-of-Flight (ToF) non-contrast MRA pulse sequences, followed by manual intravenous administration of a gadolinium-containing contrast agent and subsequently, acquisition of post-contrast MRA pulse sequences. Both sequences were compared using a visual rating scale by two experienced raters, length of the visualised LSAs and signal-to-noise ratios (SNR). This study was approved by the Institutional Review Board of East of England - Cambridge Central Research Ethics Committee. Written informed consent was obtained

Findings
CE MRA improved the visualisation of LSAs when compared with ToF MRA. The Median Visibility and Sharpness score was higher (2.75 vs 1.75; P=0.0008), the length of visualised LSAs was longer (24.4±4.5 vs 21.9±4.0 mm; P=0.01), and mean signal-to-noise ratio was higher (40.7±15.2 vs 38.9±16.1; P=0.08) in CE MRA when compared with ToF MRA. None of the participants needed sedation and one participant needed verbal reassurance to complete the scan.

Interpretation
We demonstrated that the use of CE MRA provides improved visualisation of LSAs when compared with ToF MRA at 7T. Hence, CE MRA could be very useful for research in patients with strokes due to small vessel occlusion. Although limited by a small sample size and stringent safety restrictions, this study is the first prospective study comparing CE MRA with non-contrast MRA at ultrahigh-field 7T MRI to our knowledge. Also, the scanning procedure was well tolerated in this age group, which suggests likelihood of a successful outcome in a larger study with appropriate patient selection.

D5 | Dr Abidemi Otaiku

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Dream Content Predicts Motor and Cognitive Decline in Parkinson's Disease

Background
Dream content alterations in Parkinson's disease (PD) are associated with motor and cognitive dysfunction cross-sectionally. Although recent studies suggest abnormal dream content in PD might also predict cognitive decline, the relationship between dream content and motor decline in PD remains unknown. This study investigated whether abnormal dream content in PD predicts both motor and cognitive decline.

Methods
Data were obtained from the Parkinson's Progression Markers Initiative database. Patients were evaluated at baseline and 60-months, with validated clinical scales, including the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ), Montreal Cognitive Assessment (MoCA), and the Movement Disorder Society-Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III). Patients were dichotomized using RBDSQ item 2, which inquires whether they frequently experience aggression in their dreams. Regression analyses were used to assess whether frequent aggressive dreams at baseline predicted longitudinal changes in MDS-UPDRS III and MoCA scores as well as progression to Hoehn and Yahr stage 3 (H&Y ≥3) and cognitive impairment.

Findings
Of the patients, 58/224 (25.9%) reported frequent aggressive dreams at baseline. Aggressive dreams predicted a faster increase in MDS-UPDRS III scores (β = 4.64; P = 0.007) and a faster decrease in MoCA scores (β = -1.49; P = 0.001). Furthermore, they conferred a 6-fold and 2-fold risk for progressing to H&Y ≥3 (odds ratio [OR] = 5.82; P = 0.005) and cognitive impairment (OR, 2.35; P = 0.023) within 60 months. These associations remained robust when adjusting for potential confounders.

Interpretation
This study demonstrates for the first time that frequent aggressive dreams in newly diagnosed PD may independently predict early motor and cognitive decline.

D6 | Dr Hannah Panayiotou

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Comprehensive neonatal cardiac, feed and wrap, non-contrast, free-breathing CS 4D Flow MRI assessment; a neonatal cohort study

Background
Cardiac MRI imaging is a vital tool in diagnosis, monitoring and treatment of congenital cardiac disease but long acquisition time has limited its use in the neonatal population. Technological advances in 4D Flow MRI have now made this a clinically viable option. However, the method has not been prospectively validated in neonates. We aimed to test if non-contrast compressed sensing (CS) 4D Flow MRI is feasible, and comparable to standard 2D phase contrast (PC), in the neonatal population.

Methods
This prospective cohort study recruited 14 well neonates. Non-contrast, 2D PC and CS 4D Flow MRI sequences were acquired on a Siemens 3T Prisma scanner using feed and wrap technique; five neonates were scanned in an open-top bassinet and nine in a dedicated MRI-compatible incubator. Aortic 2D PC and aortic, pulmonary trunk and superior vena cava CS 4D Flow MRI were quantified using commercially available software. Flow comparison was analysed by t-test, inter- and intra-observer agreement and internal consistency by intraclass correlation co-efficient. Parents gave informed consent. The ethics committee Yorkshire & The Humber - Leeds East (18/YH/0439) approved the study.

Findings
All neonates scanned in the incubator completed the protocol (9/9); for those in the bassinet, 3/5 neonates woke-up before protocol completion. Average total scan time was clinically acceptable (22 minutes, range 18-25). There were no adverse events for either group. Ascending aortic forward flow measured by CS 4D Flow MRI was comparable to standard 2D PC; mean forward flows were not significantly different between the methods (4.9mL Vs 4.8mL, p=0.17) with a minimal bias of 0.11mL. There was excellent inter- and intra-observer agreement for each vessel. Internal consistency measures also showed excellent agreement; main pulmonary artery forward flow and the sum of left and right pulmonary arteries had a mean difference of 0.4mL±0.7 (ICC 0.95), and ascending aorta forward flow and the sum of superior vena cava and descending aorta had a mean difference of 0.6mL±2.5 (ICC 1.00).

Interpretation
We have demonstrated that sedation and contrast-free, feed and wrap, CS 4D Flow MRI is feasible and well tolerated in neonates. Our results demonstrate that CS 4D Flow MRI is comparable to validated 2D PC imaging, with no significant difference in mean forward flow (p=0.17), and is clinically reproducible, with excellent observer agreement and internal consistency. Although the sample was relatively small this is not out of keeping with other validation studies. Further work on a larger sample is required to ensure high-quality images are reproducible and measures of agreement are robust in neonates with an array of congenital cardiac diagnoses.

D7 | Dr Thomas Poundall

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Diagnostic performance of the ABC/2 method for assessing haematoma expansion in spontaneous intracranial haemorrhage

Background
ABC/2 is a quick and well-established method for estimating intracerebral haematoma volume. Change in ABC/2 has been used to estimate haematoma expansion in clinical trials, but there is minimal evidence validating ABC/2 for detecting haematoma expansion specifically. We use data from the Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH-2) trial to establish diagnostic performance of ABC/2 for detecting haematoma expansion in comparison to gold standard semi-automated segmentation (SAS) volume calculation. We evaluate the impact of baseline haematoma characteristics on diagnostic performance.

Methods
Relative and absolute change in haematoma volume was derived from haematoma volume measurements using ABC/2 and semi-automated segmentation on baseline and 24-hour computed tomography scans. Correlation was calculated between ABC/2 and SAS-derived haematoma volume change. Diagnostic accuracy of ABC/2 for detecting haematoma expansion (>6ml absolute and/or >33% relative increase) was calculated compared to SAS. Effect of haematoma size, location and irregularity on diagnostic accuracy was tested. A Bland-Altman analysis of agreement between ABC/2 and SAS was performed. Participants undergoing surgical haematoma evacuation between scans were analysed separately to investigate the ability of ABC/2 to detect haematoma volume reduction.

Findings
Based on measurements from 2063 non-surgical participants, correlation coefficients between ABC/2 and SAS-derived haematoma volume change were $R^2 = 0.95$ (relative) and $R^2 = 0.75$ (absolute). In 48 surgical participants ABC/2 and SAS-derived absolute volume reduction correlated ($R^2 = 0.93$). ABC/2 had overall accuracy of 0.82 for detecting haematoma expansion and did not depend on baseline haematoma irregularity but was lower for haematoma >20mls and for infratentorial haematomas.

Interpretation
ABC/2-derived change in haematoma volume correlates closely with SAS-derived volume change for both surgical and non-surgical patients. ABC/2 detects haematoma expansion with good diagnostic accuracy but performance is worse for large haematomas. This suggests a future role for ABC/2 calculations to define patients with expanding haematomas quickly at the bedside who may benefit from intervention. A strength of this study is the large, international, multi-centre dataset used to investigate the real world performance of ABC/2 vs gold standard SAS techniques in a clinical trial.

D8 | Dr Sean Tan

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Structural and functional thalamic changes in Progressive Supranuclear Palsy

Background

Progressive supranuclear palsy (PSP) is a tauopathy causing motor and cognitive impairment. The thalamus is an important node in brain networks supporting cognitive and motor functions that may be relevant in explaining clinical severity in PSP.

Neuroimaging studies have largely focused on PSP -Richardson's Syndrome (RS). Thalamic atrophy alongside structural and functional connectivity alterations are a consistent finding.

The clinical impact of thalamic alterations in PSP subtypes is not known. This study examined thalamic changes across PSP phenotypes investigating (i) thalamic atrophy (ii) thalamic functional connectivity and (iii) the relationship between thalamic structural and functional connectivity changes with clinical severity.

Methods

Participants

92 participants with PSP [63 PSP-RS, 24 PSP-cortical, 5 PSP-subcortical] and 104 controls were recruited from the Cambridge Centre for Parkinson's Plus Disorders cohort. Clinical assessments and imaging were conducted within 1 year of diagnosis.

Structural Analysis

Thalamic volumes (TVs) were obtained using FreeSurfer. Bayesian multiple regression (brms, R) was used to model (i) mean TVs and inter-group differences (ii) relationships between Z-standardised clinical scores and TVs.

Functional Analysis

Voxel-wise seed-based functional connectivity of the thalamus used FSL-FEAT. Inter-group differences and relationships between clinical scores and functional connectivity were assessed using a general linear model.

Findings

TVs for all PSP subgroups were smaller than controls. No differences between PSP subgroups were detected. There was evidence TVs positively predicted Revised Addenbrooke's Cognitive Examination (ACER) scores for the entire PSP group [β = 0.28, 95% credible interval (CI) = 0.04 – 0.53)]. Subgroup analysis showed evidence for a relationship between ACER scores and TVs in PSP-RS [β = 0.33, 95% CI = 0.09 – 0.57] and PSP-cortical [β = 0.46, 95% CI = 0.12 – 0.83] phenotypes. TV loss was related to worse disease severity (measured by total PSP rating scale scores) for the PSP cohort as a whole [β= -0.51, 95% CI = -1.00 – -0.02].

PSP patients showed decreased functional connectivity in higher cortical regions with distinct distributions and magnitudes for different subgroups. Increased connectivity with the middle temporal gyrus correlated with ACER scores for PSP patients as a group and the PSP-cortical phenotype.

Interpretation

Limitations include (i) a cross-sectional design (ii) using a seed-based rsfMRI analysis which has drawbacks due to the a priori choice of regions to correlate with the rest of the brain. (iii) The thalamus as a whole was used as the seed with no distinction being made between the various components and nuclei.

In summary, thalamic volume loss is a prominent aspect of PSP, and is associated with a wide network of changes in functional connectivity that may be distinct between PSP subtypes. Changes in thalamic structure and function predict clinical severity, particularly in PSP-RS and PSP-cortical subtypes.

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