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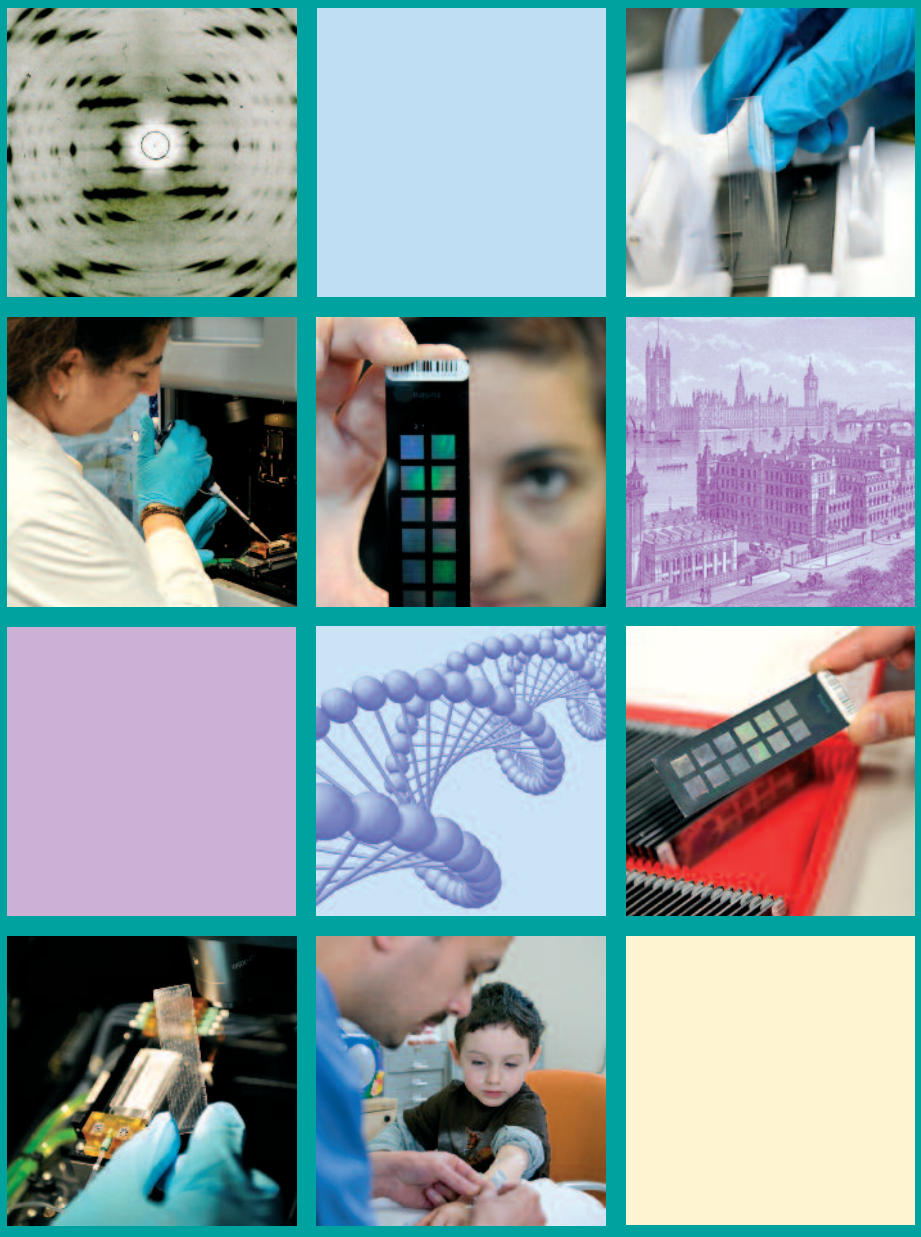
Functional Genomics Workshop



6–8 February 2013

Introduction

Functional Genomics Workshop

6-8 February 2013
St Thomas' Hospital Governors' Hall
King's College London
London, UK

Aims of the workshop

- To highlight new advances in understanding the functional impact of genetic variation in immune mediated inflammatory and rheumatic diseases.
- To provide delegates with an experimental framework for investigating the function of allelic variants.
- To provide opportunities for investigators and their collaborators to network in this field of research.

Organising Committee

Tim Vyse (KCL)
Lars Klareskog (Karolinska Institute)
Tom Huizinga (Leiden University Medical Centre)
Jane Worthington (Manchester University)
Frank Nestle (KCL)
Michael Malim (KCL)
Andrew Cope (KCL)

Workshop Sponsors

Guy's and St Thomas' NHS Foundation Trust
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Biomedical Research Centre
Working together to deliver better health through research

Be the Cure FOR RHEUMATOID ARTHRITIS

THE FRANCIS CRICK INSTITUTE

King's Bioscience Institute

KING'S College LONDON

Workshop programme

Keynote Lectures: 45 mins
Plenary Talks: 25 mins
+ 5 min discussion
Abstract presentations: 10 mins
(including discussion)

Day 1: Wednesday 6th February

13:00
Registration opens in Governor's Hall, St Thomas' Hospital

14:50
Welcome and Opening Remarks (Tim Vyse)

Session 1
15:00 – 18:00
Rheumatoid Arthritis (Chair: Jane Worthington)

Fina Kurreeman (Leiden University Medical Centre, The Netherlands):
Large scale candidate gene sequencing studies in complex polygenic disease

Stephen Eyre (University of Manchester, UK)
Insights from ImmunoChip analysis

Robert Plenge (Harvard Medical School, Boston, US)
Functional genomic analysis of the *CD40* locus

16:30 – 17:00 Tea break with poster viewing

Lars Klareskog (Karolinska Institute, Sweden)
The challenge: Parallel analysis of specificity and signalling in single B and T cells

17:30 – 18:00
Selected abstract presentations (10 min each, including discussion)

A1 Karine Chemin: Characterization of anti-collagen II autoreactive CD4+ T cells

A2 Hayley Evans: TNF-alpha blockade promotes human T helper cell plasticity through induction of IL-10: a novel role for Aiolos (IKZF3)

A3 Tom Huizinga: SPAG16 variants in joint damage progression in rheumatoid arthritis

Session 2
18:00 – 18:45
Keynote Lecture

Sponsored by King's Bioscience Institute (Chair: Lars Klareskog)

Peter Gregersen (The Feinstein Institute for Medical Research, New York, US)
Genetics of Autoimmunity: Defining Immune Rheostats

End of Day 1: Scientific sessions

19:00 – 21:00

Welcome Reception
Venue: St Thomas' Hospital, Central Hall

Workshop programme

Day 2: Thursday 7th February

Session 3

09:00 – 10:30

Connective tissue diseases I (Chair: Tom Huizinga)

Tim Vyse (King's College London, UK)
Genetic wiring in SLE – many leads to follow

09:30

Selected abstract presentations (10 min each, including discussion)

A4 Michelle Fernando: Variant HLA amino acid analysis in systemic lupus erythematosus and related subphenotypes

A5 Jose-Ezequiel Martin: Seven amino acids in HLA-DRB1 and HLA-DPB1 explain the majority of MHC associations with systemic sclerosis

A6 Lina-Marcela Diaz-Gallo: Expression of two genes from the candidate locus of chromosome X in rheumatoid arthritis and systemic sclerosis

A7 Espen Hesselberg: Genetic variability in serotonin 2A receptor affects immune responses in rheumatoid arthritis

10:30 – 11:00 Coffee break with poster viewing

Session 4

11:00 – 13:00

Lessons from animal models (Chair: Andy Cope)

Rose Zamoyska (University of Edinburgh, UK):
The influence of the cytoplasmic phosphatase, PTPN22, on T cell function and autoimmunity

Chris Denton (University College London, UK)
Transgenic perturbation of TGFbeta pathways

Rudi Beyaert (Ghent, Belgium)
A20 (TNFAIP3): A molecular brake on inflammation and immunity

Selected abstract presentations (10 min each, including discussion)

A8 Martina Johannessen: Genetic mapping of arthritis in heterogeneous stock mice (HS) mice

A9 Roulis Manolis: Post-GWAS cell-specific and functional genomic approaches for the identification of novel disease pathways in animal models of IBD

A10 Lina Olsson: The role of oxygen radicals as regulators of the immune system in autoimmune diseases

13:00 – 14:00: Lunch with posters

Keynote Lectures: 45 mins
Plenary Talks: 25 mins
+ 5 min discussion
Abstract presentations: 10 mins
(including discussion)

Day 2: Thursday 7th February

Session 5

14:00 – 16:00

Psoriasis and Spondyloarthropathy (Chair Frank Nestle)

Frank Nestle (King's College London, UK)
Psoriasis: From Genes to Function and Therapy

Matthew Brown (University of Queensland, Brisbane, Australia)
From genes to the causes of ankylosing spondylitis

Selected abstract presentations (10 min each, including discussion)

A11 Francesca Capon: An in-depth molecular dissection of the major psoriasis susceptibility locus uncovers candidate susceptibility alleles within an HLA-C regulatory element

A12 Jenny Yang: The impact of IL-2 signalling on regulatory T cell function in type 1 diabetes

A13 Alka Saxena: Transcriptome sequencing in the investigation of genetic disorders

16:00 – 16:30: Tea break

Session 6

16:30 – 18:30

Novel approaches, technologies and tools I (Chair: Tim Vyse)

Michael Simpson (King's College London, UK)
Exome sequencing in Mendelian phenotypes and application in immune disorders

Enrico Petretto (Imperial College London, UK)
Systems-genetics studies of complex diseases

Julian Knight (University of Oxford, UK)
Insights into regulatory genetic variants from mapping gene expression as a quantitative trait in primary immune cell populations

Julian Downward (LRI, London, UK)
Using functional genomic approaches to unravel oncogene dependency in cancer

End of Day 2: Scientific sessions

Speakers' Dinner
(Meeting in the speaker hotel lobby at 8pm)

Free evening for all other delegates

Workshop programme

Day 3: Friday 8th February

Session 7

09:00 – 11:00

Inflammatory Bowel Disease (Chair: Graham Lord)

Jeffrey Barrett (Cambridge, UK)
The shared genetics of infectious and inflammatory diseases

Ramnik Xavier (Harvard Medical School, Boston US)
Integrative analysis of IBD genetics

Bill Cookson (Imperial College London, UK)
Asthma: genetics on either side of the mucosal barrier

Graham Lord (King's College London, UK)
Transcriptional control of mucosal immunity

11:00 – 11:30 Coffee break

Session 8

11:30 – 12:30

Metabolic Bone Disease and Pain (Chair: Matt Brown)

Fran Williams (King's College London)
Genomic approaches to pain

Stuart Ralston (University of Edinburgh)
Genetic and Environmental determinants of Paget's disease

12:30

Connective tissue diseases II

Tim Radstake (Nijmegen, The Netherlands)
From Genes to fibrosis using the full spectrum of system biology

13:00 – 14:00 Lunch with posters

Session 9

14:00 – 15:00

Novel approaches, technologies and tools II (Chair: Tim Spector)

Tim Spector (King's College London, UK)
The Use of Twins in 'Omic Research

Anne O'Garra (NIMR, London, UK)
A Systems Biology Approach to Study the Immune Response in Tuberculosis: Implications for Advancing Diagnosis and Monitoring of Drug Treatment

Session 10

15:00 – 15:45

Keynote Lecture (Chair: Mark Peakman)

John Todd (University of Cambridge, UK)
Genetically-validated intervention strategies in autoimmune diabetes

Closing remarks

DEPART (aiming to end around 4pm)

Fina Kurreeman
Leiden University Medical Centre

Largescale candidate gene sequencing studies in complex polygenic disease

Biography: During my PhD, I undertook a hypothesis-driven approach to the identification of risk genes in RA and discovered the TRAF1/C5 region as one of the few widely replicated and genome-wide significant risk factors for Rheumatoid Arthritis (RA) at that time. I reproduced this association in a family-based association study as well several other autoimmune diseases. In recognition of this work, I was granted a "Young Investigator Award" by the "the European League against Rheumatism", the organization which represents the patient, health professional and scientific societies of rheumatology of all the European nations.

During my post-doctoral research, I transitioned into cutting edge population genetics in the group of Professor Plenge at Harvard Medical School, contributing to several published genome-wide studies (GWAS). More recently, I have also been highly involved in fine-mapping studies to uncover potential functional causal variants. I have been the first to show systematically that electronic medical records (which promises to revolutionize genomic medicine in the future) can be successfully leveraged to allow meaningful genetic studies in multi-ethnic patient collections well as subsetting patients into clinically relevant subtypes. More recently, my research has led to the discovery of two novel rheumatoid arthritis loci through the utilization of large multi-ethnic populations of European, African and East Asian ancestry. In parallel, I have been investigating whether low-frequency or rare risk alleles predispose to the risk of rheumatoid arthritis by using next-generation deep re-sequencing 25 candidate genes from GWAS loci in a large number of patients and controls. I have gone from candidate gene studies to GWAS studies, subsequently largescale sequencing and now I am extremely excited at the prospect of translating these genetic discoveries into an understanding of disease pathogenesis.

Reference:

Kurreeman F*, Diogo D*, Stahl EA, Liao KP, Gupta N, Greenberg JD, Rivas MA, Hickey B, Flannick J, Thomson B, Guiducci C, Ripke S, Adzhubey I, Barton A, Kremer JM, Alfredsson L; Consortium of Rheumatology Researchers of North America; Rheumatoid Arthritis Consortium International, Sunyaev S, Martin J, Zhernakova A, Bowes J, Eyre S, Siminovitch KA, Gregersen PK, Worthington J, Klareskog L, Padyukov L, Raychaudhuri S, Plenge RM. Rare, Low-Frequency, and Common Variants in the Protein-Coding Sequence of Biological Candidate Genes from GWASs Contribute to Risk of Rheumatoid Arthritis. Am J Hum Genet. 2013 Jan 10;92(1):15-27

Fina A.S Kurreeman, Eli A. Stahl, Yukinori Okada, Katherine Liao, Dorothee Diogo, Soumya Raychaudhuri, Jan Freudenberg, Yuta Kochi, Nikolaos A. Patsopoulos, Namrata Gupta, CLEAR investigators*, Cynthia Sandor, So-Young Bang, Hye-Soon Lee, Leonid Padyukov, Akari Suzuki, Kathy Siminovitch, Jane Worthington, Peter K. Gregersen, Laura B. Hughes, Richard J. Reynolds, S. Louis Bridges, Jr., Sang-Cheol Bae, Kazuhiko Yamamoto, Robert M. Plenge. Identification of rheumatoid arthritis susceptibility loci at 1p36 and 17q12 utilizing a multi-ethnic approach. Am J Hum Genet. 2012 Mar 9;90(3):524-32

E.A. Stahl , D. Wegmann, P. Kraft, R. Chen, H.J. Kallberg, F.A.S. Kurreeman, P.I.W. de Bakker, P.K. Gregersen, L. Alfredsson, K.A. Siminovitch, J. Worthington, S. Raychaudhuri, R.M. Plenge. Polygenic modeling of genome-wide association study data reveals hidden heritability of rheumatoid arthritis risk. Nature genetics. 2012 Mar 25;44(5):483-9

Kurreeman, F., K. Liao, L. Chibnik, B. Hickey, E. Stahl, V. Gainer, G. Li, L. Bry, S. Mahan, K. Ardlie, B. Thomson, P. Szolovits, S. Churchill, S. N. Murphy, T. Cai, S. Raychaudhuri, I. Kohane, E. Karlson, and R. M. Plenge. "Genetic Basis of Autoantibody Positive and Negative Rheumatoid Arthritis Risk in a Multi-Ethnic Cohort Derived from Electronic Health Records." Am J Hum Genet 88, no. 1 (2011): 57-69.

Kurreeman, F. A., L. Padyukov, R. B. Marques, S. J. Schrodi, M. Seddighzadeh, G. Stoeken-Rijsbergen, A. H. van der Helm-van Mil, C. F. Allaart, W. Verduyn, J. Houwing-Duistermaat, L. Alfredsson, A. B. Begovich, L. Klareskog, T. W. Huizinga, and R. E. Toes. "A Candidate Gene Approach Identifies the Traf1/C5 Region as a Risk Factor for Rheumatoid Arthritis." PLoS Med 4, no. 9 (2007): e278.

Steve Eyre

Arthritis Research UK Epidemiology Unit
Centre for Musculoskeletal Research
University of Manchester
Manchester Academic Health Science Centre, Manchester, UK

Insights from ImmunoChip analysis

My research career began in 1995 when I undertook an MSc in Biotechnology at De Montfort University, Leicester. I realised the potential of genetics to make a real difference to patient care and subsequently moved to work with Prof Gillian Wallis in Manchester, who was undertaking linkage analysis studies in osteoarthritis. My work in this field led to several publications. At that point I started to work more closely with investigators at the arthritis research UK Epidemiology Unit and joined that Unit as a research assistant in 1997. Since then, I have been involved in the investigation of a range of genetic diseases, but principally in studies of rheumatoid arthritis (RA). Initially, this involved linkage studies in affected sibling pair families but now is almost exclusively association based studies. I have published widely in this field and was awarded a PhD in 2007.

More recently, my work has focussed on following up findings from genome-wide association studies in rheumatoid arthritis. I contributed to the Wellcome Trust Case control Consortium (WTCCC) study, which included samples with RA, published in Nature. Following that, I have coordinated the program of research to follow up the most associated variants in validation studies. This has led to the identification of a number of novel RA susceptibility loci including a locus on chromosome 6q23, IL2RB and loci on chromosomes 10p15 and 12q13. These findings have been published in Nature Genetics (Thomson et al 2007; Barton et al, 2008).This work has culminated in the recent Nature Genetics paper on fine mapping RA loci on the Immunochip array (Eyre et al 2012).

I am currently supervising post doctoral and PhD students to genetically annotate these findings. This involves re-sequencing, rare variant analysis, transcript profiling, methylation studies, chromatin immunoprecipitation, pathway analysis and chromosome conformation capture (3C) experiments.

Reference:

1: Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P, Zhernakova A, Stahl E, Viatte S, McAllister K, Amos CI, Padyukov L, Toes RE, Huizinga TW, Wijmenga C, Trynka G, Franke L, Westra HJ, Alfredsson L, Hu X, Sandor C, de Bakker PI, Davila S, Khor CC, Heng KK, Andrews R, Edkins S, Hunt SE, Langford C, Symmons D; Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; Wellcome Trust Case Control Consortium, Concannon P, Onengut-Gumuscu S, Rich SS, Deloukas P, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Arlsetig L, Martin J, Rantapää-Dahlqvist S, Plenge RM, Raychaudhuri S, Klareskog L, Gregersen PK, Worthington J. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. Nat Genet. 2012 Nov 11;44(12):1336-40. doi: 10.1038/ng.2462. Epub 2012 Nov 11. PubMed PMID: 23143596.

Robert M Plenge

Brigham and Women's Hospital and Harvard Medical School,
Boston, Massachusetts 02115, USA

Session 1
Day 1: Wednesday
6th February

Functional genomic analysis of the *CD40* locus

Robert is Director of Genetics and Genomics in the Division of Rheumatology, Immunology and Allergy at Brigham and Women's Hospital; Assistant Professor of Medicine at Harvard Medical School; and Associate Member of the Broad Institute of MIT and Harvard.

His research focuses on genomic aspects of immune-mediated human diseases, with particular attention to rheumatoid arthritis (RA). As part of a highly collaborative international community, his research has contributed to the discovery of >50 RA risk loci, with publications in the New England Journal of Medicine, Nature Genetics, amongst other top-tier journals. He has an active molecular immunology research laboratory, with a goal of translating genomic discoveries into biological pathways that guide high-throughput, small molecule drug screens. He leads a multi-disciplinary team that uses electronic medical records (EMR) for discovery research in immune-mediate traits, and another multi-disciplinary team to study pharmacogenomic predictors of efficacy and toxicity in RA (as part of the Pharmacogenomic Research Network, PGRN). The ultimate goal of his research is to improve the lives of patients with immune-mediated diseases such as RA.

Robert completed his MD and PhD degrees at Case Western Reserve University (thesis advisor Hunt Willard), Internal Medicine residency at University of California, San Francisco (as a Molecular Medicine Fellow), Rheumatology fellowship at Brigham and Women's Hospital, and post-doctoral research fellowship at the Broad Institute (advisor David Altshuler).

In recognition of his passion for translational immunogenomics research, Robert has received numerous awards, including: Pre-doctoral Clinical Award from The American Society of Human Genetics (1995); The Young Investigator Award from the Department of Medicine at Brigham and Women's Hospital (2008); Burroughs Wellcome Fund Career Award for Medical Scientists (2008); and election to The American Society for Clinical Investigation (2012).

He lives with his wife and three daughters in Wellesley, MA. He enjoys skiing, socializing with friends and family, and participating in any activities that his kids want to do.

Lars Klareskog
Karolinska Institute,
Sweden

The challenge: Parallel analysis of specificity and signalling in single B and T cells

Abstracts

A1 Karine Chemin: *Characterization of anti-collagen II autoreactive CD4+ T cells*

Authors
Karine Chemin, Anca Catrina, Leonid Padyukov, Lars Klareskog, Vivianne Malmström

Affiliation
Rheumatology Unit, Dept of Medicine, Karolinska Institute, Stockholm, Sweden

Abstract text

Antibodies against the cartilage protein collagen type II (CII) are present in sera and synovial fluids from RA patients. Some of them are directed against the native protein, while others are directed against citrullinated CII. Furthermore, MHC class II restricted CII T cells epitopes have since long been identified in the context of human HLA-DR4 and mouse I-Aq, but none of these T cell peptides are citrullinated. We postulate that some RA-associated HLA-molecules could present citrullinated CII peptides.

We aimed to identify and perform functional characterization of anti-citrullinated CII CD4+T cells from RA patients. We screened the entire CII protein for peptides capable of binding RA-associated HLA molecules (ProImmune Reveal assay). Four unique citrullinated type II collagen peptides binding to HLA-DR*1001 were identified. We screened these citrullinated peptides and their unmodified counterparts in functional T-cell assays utilizing PBMC from HLA-matched RA patients and healthy donors. In PBMC from RA patients, one citrullinated CII-peptide was found to induce a specific response, as it could be blocked by anti-DR antibodies. The native peptide did not elicit any responses.

To what extent and by which mechanisms the presence of PTPN22 1858T gene variant affect the outcome of specific immune response is currently a subject of debate. In our HLA-DR10 system, we will further compare the functionality of T cells carrying or not PTPN22 risk alleles. In parallel, CD4+ T cells will be activated with a previously characterized HA (Influenza Hemagglutinin) peptide.

We have identified a novel citrullinated CII derived T cell epitope which is not presented by HLA-DR4 but by HLA-DR10. We will include the analysis of the contribution of PTPN22 risk alleles in our future efforts with the overall aim of understanding which aberrant immune responses may precipitate into RA.

A2 Hayley Evans: *TNF-alpha blockade promotes human T helper cell plasticity through induction of IL-10: a novel role for Aiolos (IKZF3)*

Authors
Hayley G. Evans, Nicola J. Gullick, Gina J. Walter, Urmas Roostalu, Klaus S. Frederiksen, Jens G. Gerwien, Andrew P. Cope, Frederic Geissmann, Bruce W. Kirkham and Leonie S. Taams

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4. Academic Department of Rheumatology, King's College London, UK
5. Department of Rheumatology, Guy's and St Thomas' NHS Foundation Trust, London, UK

Abstract text

TNF-alpha inhibitor (TNFi) therapy has revolutionized the treatment of rheumatoid arthritis (RA). IL-17-producing CD4+ T cells (Th17 cells) are considered important contributors to RA pathogenesis. Counter-intuitively, patients with RA on TNFi therapy showed an enrichment of Th17 cells in peripheral blood compared to those on disease-modifying anti-rheumatic drugs or healthy controls. However, these patients also showed an increase in IL-10 producing CD4+ T cells. In vitro addition of TNFi drugs (infliximab, adalimumab, etanercept) to human monocyte/CD4+ T cell co-cultures recapitulated the enrichment in IL-17+ and IL-10+ CD4+ T cells and revealed IL-10 co-expression in IL-17+, as well as in IFN-gamma+ and TNF-alpha+ CD4+ T cells. The induction of IL-10 expression in Th17 cells was FcgR, IL-10 and regulatory T cell-independent, occurred in memory CD4+ T cells, was functionally active, and was strongly associated with expression of the transcription factor IKZF3, encoding Aiolos. IKZF3 overexpression in primary memory CD4+ T cells enhanced IL10 expression and IL10 was reduced in CD4+ T cells from healthy donors homozygous for inflammatory disease-associated polymorphisms at the IKZF3 locus. Our data indicate that TNF-alpha blockade induces IL-10 expression in human CD4+ T cells, including Th17 cells, and reveal a novel role for Aiolos in this process.

Funded by IMI BTCURE and NIHR BRC at KCL & GSTT

A3 Tom Huizinga: *SPAG16 variants in joint damage progression in rheumatoid arthritis*

Authors

Rachel Knevel, Kerstin Klein, Klaartje Somers, Caroline Ospelt, Jeanine J. Houwing-Duistermaat4, Jessica A.B. van Nies, Diederik P.C de Rooy, Laura de Bock, Fina A.S. Kurreeman, Joris Schonkeren, Gerrie Stoeken-Rijsbergen, Quinta Helmer, Michael P.M. van der Linden, Marlena Kern, Nataly Manjarrez-Orduno, Luis Rodriguez-Rodriquez, Piet Stinissen, Tom W.J. Huizinga, Rene E.M. Toes, Steffen Gay, Peter K. Gregersen, Veerle Somers & Annette H.M. van der Helm-van Mil

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4. Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, The Netherlands;
5. Feinstein Institute for Medical Research and North Shore–Long Island Jewish Health System, Manhasset, New York, USA;
6. Dept of human genetics, Leiden University Medical Center, The Netherlands

Abstract text

Background

Joint destruction is a hallmark of auto-antibody positive Rheumatoid Arthritis (RA), though the severity is highly variable between patients. The processes underlying these inter-individual differences are incompletely understood.

Methods

We performed a genome-wide association study on the radiologic progression rate in 384 auto-antibody positive RA-patients. In stage-II 1,557 X-rays of 301 Dutch auto-antibody positive RA-patients were studied and in stage-III 861 X-rays of 742 North-American auto-antibody positive RA-patients. Spermatocyte Associated Antigen 16 (SPAG16) expression in RA synovium and fibroblast-like synoviocytes (FLS) was examined. FLS contribute to joint damage by the secretion of metalloproteinases. SPAG16 genotypes were related to MMP-3 and MMP-1 expression by FLS in vitro and MMP-3 production ex vivo.

Results

A cluster of SNPs at 2q34, located at SPAG16, associated with the radiological progression rate with rs7607479 reaching genome-wide significance. A protective role of rs7607479 was replicated in European and North-American RA-patients. Per minor allele, patients had a 0.78-fold (95%CI 0.67-0.91) progression rate over 7-years. mRNA and protein expression of SPAG16 in RA synovium and FLS was verified. FLS carrying the minor allele secreted less MMP-3 ($P=1.60x10-2$). Furthermore, RA-patients carrying the minor allele had lower serum levels of MMP-3 ($P=4.28x10-2$). In a multivariate analysis on rs7607479 and MMP-3, only MMP-3 associated with progression ($P=2.77x10-4$), suggesting that the association between SPAG16 and joint damage is mediated via an effect on MMP-3 secretion.

Conclusion

Genetic and functional analyses indicate that SPAG16 is protective against joint destruction in auto-antibody positive RA. This may represent a new target for therapeutic interventions.

Peter K Gregersen

Robert S. Boas Center for Genomics and Human Genetics
Feinstein Institute for Medical Research
North Shore LIJ Health System
Manhasset, NY

Genetics of Autoimmunity: Defining Immune Rheostats

Peter K Gregersen received his MD from Columbia University College of Physicians and Surgeons in 1976, and completed a rheumatology fellowship at the Hospital for Joint Diseases in 1984. During the 1980's, Dr. Gregersen cloned and sequenced the major HLA class II alleles associated with rheumatoid arthritis (RA) and first described the "shared epitope" hypothesis to explain the complex pattern of HLA associations with RA. In the 1990's he founded the North American Rheumatoid Arthritis Consortium (NARAC) which has made a major contribution to identifying risk alleles associated with rheumatoid arthritis. Dr. Gregersen has collaborated extensively on genetic studies of many other human autoimmune disorders, and is also pursuing genetic studies of several neurocognitive traits including absolute pitch and synesthesia. In 2007 Dr. Gregersen was the recipient of the Klemperer Medal from New York Academy of Medicine as well as the Distinguished Basic Investigator Award from the American College of Rheumatology. He was elected to membership in the Association of American Physicians in 2009.

Tim Vyse

King's College London
London, UK

Session 3
Day 2: Thursday
7th February

Genetic wiring in SLE – many leads to follow

I trained in Natural Sciences at Trinity Hall, Cambridge and then clinical medicine at Guy's Hospital Medical School. I undertook further clinical training in London at the National Hospital, Queen Square and Hammersmith Hospital and then a PhD at University of London. My post-doctoral training was at the National Jewish Center for Immunology in Denver, Colorado. I was thereafter awarded a Wellcome Trust Senior Clinical Fellowship, during which I worked at Imperial College London, becoming Professor of Rheumatology. I moved to King's in 2010, having obtained a personal Chair in Molecular Medicine.

Research interests

- Genetic basis of the autoimmune disease, systemic lupus erythematosus (SLE)
- Molecular immunology of systemic autoimmunity
- How differences in ancestry influence genetic factors in SLE
- Diagnosis and prognosis in SLE

A4 Michelle Fernando: *Variant HLA amino acid analysis in Systemic Lupus Erythematosus and related subphenotypes*

Authors
Kimberly E Taylor, David L Morris, Joanne Nititham, Sharon Chung, SLEGEN, Timothy J Vyse, Lindsey A Criswell, Michelle M A Fernando

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Abstract text

Objectives
To determine whether variant amino acids encoded by classical HLA alleles better explain MHC association signals in SLE and related subphenotypes compared to MHC region SNPs and HLA alleles alone in European populations.

Methods
We performed a meta-analysis of the major histocompatibility complex (MHC) region in four SLE cohorts of European ancestry comprising 3153 cases and 9782 healthy controls. Each cohort had high density SNP typing at the MHC and associated subphenotype information. We imputed SNP genotypes in each dataset prior to meta-analysis due to insufficient marker overlap between cohorts. We imputed 4-digit HLA allele genotypes and HLA amino acid sequence data in each cohort. We used a number of starting models (including SNP, HLA allele and HLA amino acid data alone or in combination) to perform stepwise forward and backward logistic regression in order to ascertain the best explanatory model using the BIC (Bayesian Information Criterion) in SLE and related subphenotypes. Specifically we investigated association in three groups: (i) SLE cases compared with healthy controls, (ii) specific SLE subphenotypes compared with healthy controls (anti-Ro/SSA antibody positive, anti-La/SSB antibody positive and cases with renal disease), (iii) a case only study, i.e. subphenotype positive cases compared with subphenotype negative cases.

Results
Our preliminary results demonstrate independent association of HLA-DPB1 with SLE. Furthermore, for the first time we show that specific HLA-DPB1 alleles and amino acids are associated with subsets of SLE patients' positive for anti-Ro and anti-La antibodies but not with renal disease. In addition, we find association at HLA-DRB1*03:01, HLA-DRB1*15:01 and class I markers in anti-Ro antibody positive SLE cases compared with controls. In anti-La antibody positive SLE cases compared with controls, we find association with HLA-DRB1*03:01, HLA-DPB1 and extended class I variants. In SLE patients with renal disease we find association with HLA-DRB1*15:01 and class I amino acid variants.

A5 Jose-Ezequiel Martin: *Seven amino acids in HLA-DRB1 and HLA-DPB1 explain the majority of MHC associations with systemic sclerosis*

Authors
Jose-Ezequiel Martin, Paul I.W. de Bakker, Carmen P. Simeon, Norberto Ortego-Centeno, Patricia Carreira, Miguel A. Gonzalez-Gay6, Nicolas Hunzelmann, Madelon C. Vonk, Annemie J. Schuerwegh, Alexandre E. Voskuyl, Gabriela Riemekasten, Torsten Witte, Olga Gorlova, Frank C. Arnett, Xiaodong Zhou, Shervin Assassi, John D. Reveille, Timothy R.D.J. Radstake, Maureen D. Mayes, Javier Martin, Bobby P.C. Koeleman.

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14. Department of Epidemiology, M.D. Anderson Cancer Center, Houston, Texas, USA.
15. The University of Texas Health Science Center–Houston, Houston, TX, USA.
16. Department of Rheumatology and Clinical Immunology, Utrecht Medical Center, The Netherlands.

Abstract text
Distinct HLA alleles are associated with systemic sclerosis (SSc), but they collectively do not explain the strong association signal observed for the HLA region in recent genome wide association studies. Here, we took advantage of existing dense genotype data and imputed the HLA class I and II alleles, together with 894 polymorphic aminoacidic positions and 3,841 SNPs, in 2,296 cases and 5,356 controls of European origin. Conditional analyses revealed distinct signatures of association within SSc subtypes related to two auto-antibodies anti-centromere (ACA) and anti-topoisomerase I (ATA) status. Three variable aminoacids in positions 13, 60 and 71 of the HLA-DR 1 molecule explain the majority of

HLA association with ACA. Similarly, variable aminoacids at position 76 of the HLA-DP 1 molecule and 58, 67 and 86 of the HLA-DR 1 molecule explain the majority of association towards ATA production. No significant association remains after controlling for these two groups of aminoacids. These results suggest that the HLA association with SSc is different between the autoantibody subgroups and is determined by the two groups of aminoacids.

A6 Lina-Marcela Diaz-Gallo:
Expression of two genes from the candidate locus of chromosome X in rheumatoid arthritis and systemic sclerosis

Authors
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Cellular Biology and Immunology Department, Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Granada, Spain.

Abstract text

Background
There are increasing evidence of association between variations at Xq28 genomic region and autoimmune diseases. The genes interleukin-1 receptor-associated kinase 1 (IRAK1) and methyl-CpG-binding protein 2 (MECP2) are located there.

Aim
To evaluate whether there are differential mRNA expression of the IRAK1 and MECP2 between rheumatoid arthritis (RA) patients, systemic sclerosis (SSc) patients and healthy controls.

Methods
The gene expression of IRAK1 and MECP2 was measured using quantitative PCR by TaqMan assays. We studied 50 RA patients, 35 SSc patients and 52 healthy controls from a Caucasian population, all females.

Results
The IRAK1 and MECP2 expression was significantly different between the RA patients, SSc patients and controls ($p=0.0003$;$p=0.0006$,respectively,Kruskal-Wallis test). When we compared each group of patients against controls we observed that the IRAK1 expression was significantly decreased in the RA patients compared with controls ($p=0.0022$;RA relative quantity (RQ) mean=42.37; controls RQ mean=60.28, Mann-Whitney test). But there was no significant difference between the levels of IRAK1 expression between SSc patients and controls ($p=0.39$; SSc RQmean=46.8;controls RQmean=42.12). We found no significant difference in the MECP2 expression levels between RA patients and controls ($p=0.05$; RA RQmean=45.72; controls RQmean=57.06). Meanwhile, the SSc patients exhibited a significant increased expression level of MECP2 compared with controls ($p=0.017$; SSc RQmean=51.86; controls RQmean=38.71). Finally, we observed that there was a moderated positive correlation between the IRAK1 and MECP2 expression in the studied individuals (correlation coefficient=0.613, $p<0.0001$).

Conclusion
Our study is the first insight into the functional role of the IRAK1 and MECP2 genes in RA and SSc. The results showed an altered expression of these genes compared to the controls. With this study we increase the evidence that suggest an important pathogenic role of this locus at chromosome X in autoimmune diseases.

A7 Espen Hesselberg: *Genetic variability in serotonin 2A receptor affects immune responses in rheumatoid arthritis*

Authors
Snir, Hesselberg, Amoudruz, Klareskog,
Zarea-Ganji, Catrina, Padyukov, Malmström, Seddighzadeh

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Abstract text
Many genetic variants associate with the risk of developing rheumatoid arthritis (RA); however, their functional roles are largely unknown. Here, we aimed to investigate whether the RA-associated serotonin receptor 2A (HTR2A) haplotype affects T-cell and monocyte functions. Patients with established RA (n=379) were genotyped for two single-nucleotide polymorphisms (SNPs) in the HTR2A locus, rs6314 and rs1328674, to define presence of the risk haplotype for each individual. Patients with and without the RA-associated TC haplotype were selected and T-cell and monocyte function was monitored following in vitro stimulations with staphylococcal enterotoxin B and lipopolysaccharide (LPS) using multiparameter flow cytometry. Within the cohort, 44 patients were heterozygous for the TC haplotype (11.6%) while none were homozygous. Upon stimulation, T cells from TC-carrier patients produced more proinflammatory cytokines (tumor necrosis factor alpha (TNF-), interleukin-17 (IL-17) and interferon gamma (IFN-)) and monocytes produced higher levels of TNF- compared with patients carrying the non-TC haplotype (P<0.05 and 0.01, respectively). Such cytokine production could be inhibited in the presence of the selective 5-HT2 receptor agonist (2,5-Dimethoxy-4-iodoamphetamine, DOI); interestingly, this effect was more pronounced in TC carriers. Our data demonstrate that association of RA with a distinct serotonin receptor haplotype has functional impact by affecting the immunological phenotype of T cells and monocytes.

Rose Zamoyska
Institute of Immunology and Infection Research
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The influence of the cytoplasmic phosphatase, PTPN22, on T cell function and autoimmunity

Rose Zamoyska has been Professor of Immune Cell Biology at the University of Edinburgh since 2007. She obtained her PhD from the University of Cambridge in 1981. After a postdoctoral fellowship in Stanford, USA she moved to London where she established her research group first at University College and then in 1992 as a tenured scientist at the National Institute for Medical Research in Mill Hill. The primary interest in the lab is in the proximal signalling events downstream of T cell receptor engagement and developing an understanding of how these signals regulate T cell behaviour. Using genetically manipulated mouse models our current research focuses on how changes in key proximal signalling molecules influence T cell homeostasis, responses to pathogens, and the development of autoimmunity.

Reference:

The key reference is: Brownlie, R.J., L.A. Miosge, D. Vassilakos, L.M. Svensson, A. Cope, and R. Zamoyska. 2012. Lack of the Phosphatase PTPN22 Increases Adhesion of Murine Regulatory T Cells to Improve Their Immunosuppressive Function. Science Signaling 5:ra87

Christopher Denton

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London, UK

Session 4
Day 2: Thursday
7th February

Transgenic perturbation of TGFbeta pathways

Christopher Denton is Professor of Experimental Rheumatology at UCL and Consultant Rheumatologist at the Centre for Rheumatology, Royal Free Hospital, London. Professor Denton's laboratory research has focused on mediators of vasculopathy and fibrosis in connective tissue disease and preclinical models. He leads the large clinical programme in scleroderma at the Royal Free Hospital and co-ordinates multidisciplinary care for more than 1200 patients. He studied medicine at Guy's Hospital in London, where he qualified with distinction and later obtained a PhD from University College London. Following a Wellcome Trust Advanced Fellowship in molecular genetics at the M.D. Anderson Cancer Center in Houston, USA, he was appointed as a senior lecturer at UCL and consultant in rheumatology at the Royal Free Hospital in 2000, and promoted to professor in 2006. He currently chairs the UK Scleroderma Study Group (UKSSG), is Treasurer of EUSTAR (EULAR Scleroderma Trials and Research group) and past-President of the Scleroderma Clinical Trials Consortium (SCTC). He is chair of the Heberden Scientific Committee of the British Society for Rheumatology (BSR) and has published extensively on laboratory and clinical aspects of connective tissue disease and pulmonary hypertension, including novel genetic models.

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Rudi Beyaert

Department of Biomedical Molecular Biology
Ghent University
Ghent, Belgium

A20 (TNFAIP3): "a molecular brake on inflammation and immunity"

Rudi Beyaert is full professor in molecular biology at the University of Ghent (Belgium) and Associate Director of the Department for Molecular Biomedical Research of the VIB research institute. He is heading the Unit of Molecular Signal Transduction in Inflammation, whose mission is to study the molecular mechanisms that control initiation, progression and resolution of inflammation and immunity. More specifically he investigates signal transduction pathways and responses that are triggered by specific cytokine receptors (TNF, IL-1, IL-33), pattern recognition receptors (TLRs, RLRs), and T cell receptors. Major contributions to the field include the characterization of the ubiquitin-editing protein A20 (TNFAIP3) as a key regulator of innate immunity and inflammation, the identification of ABINs as ubiquitin-binding and NF- B regulatory proteins, and the discovery of the paracaspase MALT1 as a novel protease and therapeutic target in T and B cells. Currently his group is trying to further understand the mechanism of action, regulation and physiological role of these molecules using a variety of biochemical, molecular and cellular approaches combined with mouse gene targeting and mouse models of human disease. Rudi Beyaert has published over 170 papers on his research, which have been cited more than 9600 times. He received several awards, amongst which in 2012 the five-yearly Prize of Fundamental Medical Sciences of the Belgian Royal Academy of Medicine, and is on the editorial board of multiple scientific journals.

A8 Martina Johannessen: *Genetic mapping of arthritis in heterogeneous stock mice (HS) mice*

Authors
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Medical Inflammation research, Karolinska Institutet

Abstract text
Resolving the genetic basis of complex diseases like rheumatoid arthritis will require knowledge of the corresponding diseases in experimental animals to enable translational functional studies. Mapping in F2 populations has been successful but generally generate confidence intervals spanning half a chromosome. Genetic mapping in outbred mice of known ancestry is an alternative method for fine-mapping genetic loci with small phenotypic effects. The method exploits recombinants that have accumulated over many generations of outbreeding in genetically heterogeneous stocks that are derived from eight inbred strains. Because the stock has been maintained for more than 50 generations, each chromosome is a fine-grained mosaic of the progenitor strains: the average distance between recombinants is small (less than 2 centimorgans) so that the HS provide high resolution mapping of multiple genetic loci across the genome. Moreover, since they are a mosaic of eight inbred strains they are more similar to the human population but still with all the advantages of an animal model. Recent advances in genotyping technologies and analytical approaches have made it possible to use HS animals for genetic studies.

We have recently completed a study of 1800 HS mice with the major MHC gene for development of arthritis (Aq) inserted. The mice were phenotyped for the rheumatoid arthritis model collagen induced arthritis (CIA). In this unique study we showed the utility of the HS inbred-outbred cross to identify and fine-map loci affecting traits that are dependent on a specific genotype, such as MHC in this case. We mapped 26 loci with a high likelihood of affecting CIA, adding 18 new loci for CIA and were also able to fine map some of the previously known loci. Several of these loci have small numbers of candidate genes in the peak region, making them excellent starting points for positional cloning efforts.

A9 Roulis Manolis: *Post-GWAS cell-specific and functional genomic approaches for the identification of novel disease pathways in animal models of IBD*

Authors
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BSRC Alexander Fleming, Vari, Greece

Abstract text
Recent meta-analysis of GWAS for Inflammatory Bowel Diseases (IBD) revealed association with MAP3K8-TPL2 gene (1). To functionally validate the MAP3K8-TPL2 association and examine potential mechanistic interactions with other IBD-associated loci we aimed to investigate cell-specific ablation of map3k8-tpl2 gene in intestinal homeostasis through the generation of complete and conditional Tpl2 knock-out mice. We show that Tpl2 deficient mice are highly susceptible to dextran sodium sulfate-induced colitis showing enhanced loss of crypts, ulceration and lethality. Despite its well-established role as an inflammatory mediator, Tpl2 is dispensable for inflammatory infiltration upon epithelial injury. However, Tpl2 is required in mechanisms of epithelial homeostasis since Tpl2 deficient mice show defective compensatory epithelial proliferation leading to extensive epithelial depletion. These results establish a homeostatic role of Tpl2 in the gut and functionally validate GWAS findings on MAP3K8-TPL2 association with IBD. Ongoing studies using our conditional Tpl2 deficient mice (2) will identify the cell-specific basis of Tpl2 homeostatic role in potential crosstalk with other associated loci.

In a forward genetics approach we performed whole genome N-ethyl-N-nitrosourea (ENU)-induced mutagenesis in Tnf ARE/+mice which spontaneously develop Crohn-like IBD pathology due to dysregulated TNF production (3). Phenotypic screening of progeny identified a mutant line (G1) showing strong attenuation of IBD pathology. Genetic mapping performed in G3 mice using 82 genetic markers covering the whole genome revealed linkage (P=3.2e-07) ona ~17Mb region of chromosome 11containing 273 known genes, amongst them several genesrelated to immune functions such as Il12b, Il3, Il5, Il13, Il4 and miR146. Combining the results of genetic mapping analysis with whole genome sequencing of mutant mice we aim to identify candidate causative mutations for further functional analysis and validation.

A10 Lina Olsson: *The role of oxygen radicals as regulators of the immune system in autoimmune diseases*

Authors
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Abstract text
Reactive oxygen species (ROS) are generally considered to be damaging and pro-inflammatory. An increasing number of studies are however showing a different role for ROS; as regulators of the inflammatory response and protectors against the development of autoimmune disease. Extensive work in animal models have established that reduced levels of ROS enhance induced arthritis and lead to reduced immunologic tolerance A main producer of ROS is the NADPH oxidase complex (NOX2) expressed in phagocytic cells of the immune system. The NOX2 complex is made up of six genes including NCF1 and NCF4. We have previously reported that genevariants in NCF1 and NCF4 are associated with Rheumatoid Arthritis (RA) (1, 2). The genomic structure of NCF1 is complex and the RA association is with a reduced copy number of NCF1, indicating a protective effect of multiple NCF1 copies. Polymorphisms in NCF4 are also associated with Crohn's disease and recently a genevariant in the NOX2 gene NCF2 was shown to be associated with systemic lupus erythematosus. This genevariant decreases the NOX2 produced ROS, which is in line with a protective role for ROS against the development of autoimmune disease. In this study we are investigating the effect of RA associated genevariants on NOX2 produced ROS. We are measuring NOX2 ROS production in neutrophils and peripheral blood mononuclear cells, isolated from blood donated by RA patients carrying the relevant alleles. We have found that an allele in NCF1, expressed in 5% of Caucasians of Swedish origin, significantly reduces extra cellular ROS production. The next step in this study is to investigate if increased copy number of NCF1 can protect against the effect of this allele.

Frank O Nestle

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Psoriasis: From Genes to Function and Therapy

Professor Frank O Nestle holds the Mary Dunhill Chair of Cutaneous Medicine and Immunotherapy, at St. John's Institute of Dermatology, King's College London. He is a Non Executive Director at Guy's and St Thomas' Hospitals and a member of the Biomedical Research Centre (BRC) Executive. He is also Director of the Federation of Clinical Immunology Society (FOCIS) Centre of Excellence King's College London and is currently Chair of the British Society for Investigative Dermatology (BSID).

His main research interests include the pathogenesis and immunotherapy of inflammatory skin disease and skin cancer. He has given over 350 scientific lectures at national and international conferences. He was a Visiting Professor at the Mayo Clinic, Yale Medical School and Rockefeller University. He is a member of numerous national and international societies.

He has published over 150 scientific articles in publications such as Nature, Nature Medicine, New England Journal of Medicine, Journal of Experimental Medicine and Lancet. He has received numerous awards including the Alfred Marchionini Research Award at the 20th World Congress of Dermatology and the American Skin Association Achievement Award at the Tricontinental Meeting of the Societies of Investigative Dermatology

Reference:

1) Di Meglio P, Perera GK, Nestle FO. The multitasking organ: recent insights into skin immune function. Immunity. 2011 Dec 23;35(6):857-69. 2) Nestle FO, di Meglio P, Qin JZ, Nickoloff BJ. Skin Immune Sentinels in Health and Disease. Nat Rev Immunol. 2009; 9: 679-91. 3) Chu CC, Ali N, Karagiannis P, Di Meglio P, Skowera A, Napolitano L, Barinaga G, Grys K, Sharif-Paghaleh E, Karagiannis SN, Peakman M, Lombardi G, Nestle FO Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation. J Exp Med. 2012 May 7;209(5):935-45. 4) Di Meglio P, Di Cesare A, Laggner U, Chu CC, Napolitano L, Villanova F, Tosi I, Capon F, Trembath RC, Peris K, Nestle FO. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. PLoS One. 2011 Feb 22;6(2).

Matt Brown

Department of Renal Medicine and Medicine
St George Hospital and University of NSW
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Session 5
Day 2: Thursday
7th February

‘From genes to the causes of ankylosing spondylitis’

Professor Matt Brown is a clinician-scientist who trained initially in medicine and rheumatology in Sydney, Australia before moving in 1994 to Oxford, England to pursue research in genetics of bone and joint diseases, particularly ankylosing spondylitis. He was appointed Professor of Musculoskeletal Sciences at University of Oxford in 2004 and was Deputy Director of the University of Oxford Institute of Musculoskeletal Sciences from 2003-5.

In 2005 Professor Brown returned to Australia, taking a chair of Immunogenetics at University of Queensland Diamantina Institute in Brisbane. There he continues to work in genetics of common diseases, as well as running a specialist service for spondyloarthritis patients at Princess Alexandra Hospital. Professor Brown was appointed Director of The University of Queensland Diamantina Institute in 2011.

A11 Francesca Capon: *An in-depth molecular dissection of the major psoriasis susceptibility locus uncovers candidate susceptibility alleles within an HLA-C regulatory element*

Authors
Francesca Capon, Alex Clop, Anna Bertoni, Sarah L Spain, Venu Pullabhata, Michael Simpson, Adrian C Hayday, Frank O Nestle, The Wellcome Trust Case Control Consortium, Joseph F Costello, Jonathan N Barker, Richard C Trembath

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4. Queen Mary University of London, Barts and the London School of Medicine and Dentistry, London E1 2AD,

Abstract text
Psoriasis is an inflammatory skin disease that is inherited as a common and complex trait. Although genome-wide association scans (GWAS) have identified > 30 disease susceptibility intervals, more than 50% of the genetic variance is accounted for by a single Major Histocompatibility Complex (MHC) locus, known as PSORS1. HLA-C is widely regarded as the strongest PSORS1 candidate, as markers tagging HLA-Cw*0602 consistently generate the most significant signals in GWAS. At the same time, it is unclear whether HLA-Cw*0602 is itself the causal PSORS1 allele, especially as the role of SNPs that may affect its expression has not been investigated.

Here, we have undertaken detailed molecular investigation of the PSORS1 interval, with a view to identifying regulatory variants that may contribute to psoriasis susceptibility. We first analysed high-denisty SNP data and refined PSORS1 to a 179kb segment By comparing multiple MHC sequences spanning this refined locus, we identified 144 candidate susceptibility variants, which are unique to chromosomes hargbouring HLA-Cw*0602. In parallel, we examined the epigenetic profile of the critical PSORS1 interval and uncovered three enhancer elements likely to be active in T cells. Finally we showed that 9 candidate susceptibility SNPs map within a HLA-C enhancer, where three variants co-localise with binding sites for immune-related transcription factors. These data indicate that SNPs affecting HLA-Cw*0602 expression are likely to contribute to psoriasis susceptibility and highlight the importance of integrating multiple experimental approaches in the investigation of complex genomic regions such as the MHC.

A12 Jenny Yang: *The impact of IL-2 signalling on regulatory T cell function in type 1 diabetes*

Authors
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Affiliation
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Abstract text
Type 1 diabetes (T1D) is an autoimmune disease mediated by cellular destruction of insulin-producing beta cells in the pancreas. Mechanisms leading to T1D are multifactorial and depend on a complex combination of genetics, epigenetic, molecular and cellular elements that result in the breakdown of peripheral tolerance, including defective function of CD4+CD25+ regulatory T cells (Tregs). IL-2 pathway signalling is now widely known to be important in the generation and function of Tregs. As various genes in the IL-2 pathway, such as IL2, IL2RA, PTPN22 and PTPN2, have equivocally been shown to be associated with T1D and that patients with T1D have reduced IL-2 signalling in CD4+ T cells suggest that impairment of the IL-2 signalling pathway may play a role in the pathogenesis of T1D. Since administration of low dose IL-2 has been shown to improve Treg survival and function and prevent T1D onset and development in non-obese diabetic (NOD) mice, this implicates for the use of IL-2 replacement immunotherapy for the treatment and prevention of T1D. We aim to identify a subgroup of T1D patients that have a reduction in IL-2 signalling and investigate if this defect can act as a biomarker to identify patients with Treg dysfunction, and, furthermore, to see whether similar IL-2 therapy could correct this dysfunction in humans.

A13 Alka Saxena: *Transcriptome sequencing in the investigation of genetic disorders*

Authors
Alka Saxena, Annarita Patrizi, Lea Hachigian, Mari Nakamura, Nicolas Bertin, Michiel de Hoon, Erik Arner, Alistair R. R. Forrest*, Piero Carninci* and Michela Fagiolini*

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Abstract text
It is now accepted that variations in RNA expression between healthy and diseased cells contribute to disease phenotype. Depending on the amount of starting material and the length of RNA to be studied, various transcriptome-sequencing technologies are available, to identify differentially expressed RNA trancripts in cell populations. Methods for long RNA sequencing such as Cap Analysis of Gene Expression (CAGE), CAGEscan and RNA-seq require different amounts of starting material, but provide a comprehensive, semi-quantitative atlas of RNA expression in cells. Here we used CAGE to identify the gene expression changes that underlie the regression of visual function in the mouse model of Rett Syndrome. We analyzed the transcriptome of the visual cortex of Mecp2 KO mice in comparison with wild-type littermate controls at three developmental ages: eye opening (postnatal day 15; P15), peak of visual acuity (P30) and adulthood P60. Remarkably, our data reveal no differentially expressed transcripts at P15 before the onset of the visual phenotype, 54 transcripts with variable expression at P30 when the vision is still normal and 844 transcripts mis-regulated at P60, when the mutant mice show a clear impairment of visual function. Our data reveal that gene expression changes precede the onset of the visual phenotype and identify the biological pathways perturbed in the visual cortex of Mecp2 KO mice before and after the onset of symptoms. Our studies demonstrate the utility of CAGE in the investigation of genetic disorders.

Michael Simpson

Genetics and Molecular Medicine
King's College London

Disease gene discovery by exome sequencing in Mendelian phenotypes and application in immune disorders

Michael leads the Genomic Medicine Group in the Division of Genetics and Molecular Medicine at King's College London. He studied for his PhD at St George's University of London where he worked extensively on mapping of disease causing genes in isolated populations. His research led to the discovery of novel neurological disorders present at elevated frequencies in the Old Order Amish. Michael moved to KCL in 2009 where he established the rare disease exome sequencing program with Prof Richard Trembath. The program has successfully utilised next generation sequencing approaches to define the molecular genetic basis of over several inherited diseases.

Enrico Petretto

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Session 6
Day 2: Thursday
7th February

Systems-genetics studies of complex diseases

Enrico Petretto leads the Integrative Genomics and Medicine Group at the MRC Clinical Sciences Centre of Imperial College London. Enrico is a computational biologist with a background in statistical genetics, computational biology and genetic epidemiology, and is leading a research program at the interface between computational and applied statistical genetics. The aims of the Integrative Genomics and Medicine Group are: 1) to establish novel and efficient systems-genetics approaches to the regulation of complex traits and disease; 2) to identify genes and molecular networks underlying cardiovascular and metabolic traits in the rat and translate the findings into humans; 3) to discover 'master genetic regulators' of networks underlying complex phenotypes and disease to inform functional targets for more efficient drug discovery.

Reference:

SR Langley, et al. Systems-level approaches reveal conservation of trans-regulated genes in the rat and genetic determinants of blood pressure in humans. Cardiovascular Research (2012)

M Heinig*, E Petretto*, et al. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature (2010)

E Petretto*, L Bottolo*, et al. New insights into the genetic control of gene expression using a Bayesian multi-tissue approach. PLoS Computational Biology (2010)

L Bottolo, E Petretto, et al. Bayesian detection of expression quantitative trait loci hot-spots. Genetics (2011)

C McDermott-Roe, et al. Endonuclease G is a novel determinant of cardiac hypertrophy and mitochondrial function. Nature (2011)

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Julian Knight

Wellcome Trust Centre for Human Genetics
University of Oxford

Insights into regulatory genetic variants from mapping gene expression as a quantitative trait in primary immune cell populations

Julian Knight is a clinician scientist who studied Medicine at the University of Cambridge and the University of Edinburgh before completing his DPhil at the University of Oxford at the Weatherall Institute of Molecular Medicine in 1998 working with Dominic Kwiatkowski on malaria genetics. He went on to develop his research interest in transcriptional regulation and functional genomics working in Oxford at the Wellcome Trust Centre for Human Genetics and at Harvard University at the Department of Molecular and Cellular Biology with Tom Maniatis. Since 2005 he has worked at the Wellcome Trust Centre for Human Genetics as a Principal Investigator and as an Honorary Consultant Physician at the Oxford University Hospitals NHS Trust. The core interest of the lab's research is how genetic variation between individuals modulates genes critical to mounting an appropriate immune and inflammatory response and may contribute to susceptibility to autoimmune and infectious disease with a particular interest in regulatory variants involving the Major Histocompatibility Complex.

Reference:

Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A, Ellis P, Langford C, Vannberg FO, Knight JC. 2012 Genetics of gene expression in primary immune cells identifies cell-specific master regulators and roles of HLA alleles. Nature Genetics 44, 502-510

Julian Downward

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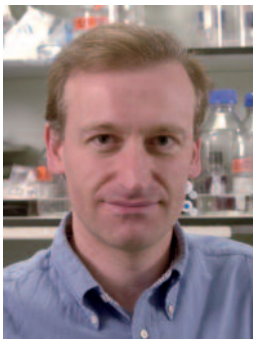
Session 6
Day 2: Thursday
7th February

Using functional genomic approaches to unravel oncogene dependency in cancer



Julian Downward obtained his bachelor's degree in Natural Sciences from Cambridge University and then studied for his Ph.D. in the laboratory of Michael Waterfield at the Imperial Cancer Research Fund in London, where he established in 1984 the link between a retroviral oncogene (v-erbB) and a cellular growth regulatory protein, the EGF receptor. In 1986, he moved to Robert Weinberg's laboratory at the Whitehead Institute at the Massachusetts Institute of Technology in Cambridge, MA, where he began work on the role of RAS proteins in human cancer. In 1989 he started his own lab at the Imperial Cancer Research Fund, now Cancer Research UK London Research Institute. His lab has provided unique insights into the molecular mechanisms of function and regulation of oncogenic proteins of the RAS family and the importance of their mutational activation in human tumours. Most recently he has focused on identifying unique weaknesses of cancer cells expressing the activated KRAS oncogene using a combination of large-scale functional genomics and pre-clinical models of lung cancer.

Julian has been Associate Director of the Cancer Research UK London Research Institute since 2005, was elected to the membership of the European Molecular Biology Organisation in 1995, was made a Fellow of the Royal Society in 2005, a Fellow of the Academy of Medical Sciences in 2009 and an honorary Fellow of the Royal College of Physicians in 2012. He belongs to the Editorial Boards of the journals Cell, Science and Molecular Cell, among others.

Reference:

1)M.S. Kumar, D.C. Hancock, M. Molina-Arcas, M. Steckel, P. East, M. Diefenbacher, E. Armenteros-Monterros, F. Lassailly, N. Matthews, E. Nye, G. Stamp, A. Behrens, J. Downward (2012) Cell 149, 642-655. "The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small celllung cancer." 2) M. Steckel, M. Molina-Arcas, B. Weigelt, M. Marani, H. Kuznetsov, G. Kelly, B. Saunders, M. Howell, J. Downward*, D. Hancock (2012) Cell Research 22, 1227-1245. "Determination of synthetic lethal interactions in KRAS oncogene dependent cancer cells reveals novel therapeutic targeting strategies." (* Corresponding author)

Jeffrey Barrett
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The shared genetics of infectious and inflammatory diseases

Jeffrey Barrett is a statistical geneticist leading a group at the Sanger Institute that uses high throughput genomic technologies to study the genetics of complex diseases of immunity and infection, including inflammatory bowel disease, type 1 diabetes and tuberculosis. He has led efforts as part of international consortia which have identified over 200 regions of the genome associated with these diseases. Before joining the Sanger Institute he worked at the Whitehead Institute in Boston, got his D. Phil at the University of Oxford, and worked as a post-doc at the University of Cambridge. He is also involved in the personal genomics movement, as an active member of Genomes Unzipped blog and having organized Sanger's "Society and the Personal Genome" program.

Ramnik J Xavier

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Session 7
Day 3: Friday
8th February

Integrative analysis of IBD genetics

Clinical gastroenterologist and molecular biologist Ramnik Xavier is Chief of Gastroenterology at Massachusetts General Hospital. The overall goal of his laboratory is to understand the function of mediators and effectors involved in innate and adaptive immunity. A second major focus is to understand the function of genes associated with Crohn's disease/ulcerative colitis and risk of autoimmunity using genomics, computational tools and model systems. Recent findings in the laboratory have contributed to elucidating the role of autophagy in Crohn's disease and the discovery of novel immune regulatory genes. In future studies, Dr. Xavier's team hopes to gain insights into the cell-cell interactions and regulatory networks that define functional modules at the host cell-microbe interface in the intestinal mucosa.

Dr. Xavier completed his clinical training in Internal Medicine, followed by subspecialty training in Gastroenterology and Hepatology, at Massachusetts General Hospital. His laboratory is located at the Center for Computational and Integrative Biology, of which he is a founding member, at Massachusetts General Hospital. Dr. Xavier is the Kurt Isselbacher Associate Professor of Medicine at Harvard Medical School, a Senior Associate Member of the Broad Institute of Harvard and MIT, and sees patients at the Crohn's and Colitis Center at Massachusetts General Hospital.

Bill Cookson

Respiratory Science Division
Imperial College London
London, UK

Asthma: genetics on either side of the mucosal barrier

Bill Cookson is Professor of Genomic Medicine and Head of the Respiratory Science Division at Imperial College London. Previously he was Professor of Human Genetics at Oxford University. He qualified in Medicine at the University of Western Australia in 1975 and trained as a Chest Physician. He completed his MD on the epidemiology of asbestosis in 1986, and a DPhil on the genetics of asthma in 1994. He was elected as a Fellow of Green College Oxford in 1996, and was awarded Fellowship of the Academy of Medical Sciences in 1999 and Fellowship of the Royal College of Physicians in 2000. Over the past 20 years the group he leads with Miriam Moffatt have found many genetic effects on asthma. They are now applying genomic technologies to other respiratory diseases, including lung cancer and respiratory infections.

Reference:

1) Moffatt MF*, Gut IG*, Demenais F*, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WOCM, and the GABRIEL consortium. A GABRIEL consortium Large-Scale Genome-Wide Association Study of Asthma. N Engl J Med. 2010. Sep 23;363(13):1211-21. 2) Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO. Disordered microbial communities in asthmatic airways. PLoS One. 2010 Jan 5;5(1):e8578.

Graham Lord

Session 7
Day 3: Friday
8th February

Professor of Medicine, King's College London
Honorary Consultant in Nephrology and Transplantation,
Guy's and St Thomas' NHS Foundation Trust
Head of Department of Experimental Immunobiology, King's College London
Director of NIHR Biomedical Research Centre, Guy's and St Thomas' Hospital and King's College London

Transcriptional control of mucosal immunity

Graham M. Lord MA, MB BChir, FSB, FRCP, PhD, qualified in medicine from Cambridge University in 1991 and undertook a period of general clinical training as a junior doctor in Cambridge, Hammersmith, Oxford and the Brompton. Following specialisation in Nephrology, Transplantation and General Medicine at the Hammersmith, he undertook a period of research in Transplantation Immunology at Imperial College London that led to a PhD in 2000, funded by an MRC Clinical Training Fellowship.

He was appointed as a Consultant in Nephrology and Transplantation at the Hammersmith in 2003. From 2003-2006, funded by an MRC Clinician Scientist award, he was a visiting scientist at Harvard University and came back to the UK to become the Chair of Medicine at King's in 2006.

Professor Lord has built up a research group at King's College London investigating fundamental immune cell biology and the translation of this knowledge to the diagnosis and treatment of patients with organ transplant rejection and autoimmune diseases. As an NHS consultant, he practices clinical nephrology with a particular interest in renal and pancreatic transplantation.

In 2011, Professor Lord led the successful application for the NIHR Biomedical Research Centre at Guy's and St Thomas' and was appointed as Director of the Centre in 2012. In this role, he is responsible for the delivery of programmes of translational research with a significant part of the Centres' portfolio focussed on Regenerative Medicine and Cellular Therapy.

Frances Williams

Department of Twin Research and Genetic Epidemiology
King's College London
London, UK

Genomic approaches to pain

Senior Lecturer and Hon Consultant Rheumatologist at the Department of Twin Research and Genetic Epidemiology, King's College London, Frances qualified in medicine with distinction from Imperial College in 1992 and undertook her PhD in the lab while training in Rheumatology. She was awarded two prizes during her training and obtained a Wellcome Trust intermediate fellowship in 2007. This facilitated the transition in research to genetic epidemiology and the study of chronic pain.

The broad interest of her group is in the genetic epidemiology of common complex traits, with the main focus on degenerative musculoskeletal diseases such as degenerative disc disease, osteoarthritis and chronic widespread pain. Such conditions provide a very high social burden in terms of direct cost to medical services and days lost from work and are relatively underfunded and undervalued in research terms. Work aims to identify genetic or epigenetic variation accounting for the expression of these phenotypes. The ultimate aim is to understand better the cellular pathways involved and thereby identify novel therapeutic targets. There are collaborations with research groups nationally and internationally and with industry: we fully support the large scale sharing of data in complex trait genetics. Future plans include translating our findings from twin volunteers to patients with chronic pain, and we have an active collection of DNA from patients with fibromyalgia from across the country as part of the BRC NIHR BioResource. FW is part of an international working party set up to standardise the definition of MR image degenerative disc phenotype for epidemiological study. She has contributed to over 40 original publications as well as reviews and book chapters.

Stuart H Ralston

Rheumatic Diseases Unit
Institute of Genetics and Molecular Medicine
University of Edinburgh, Western General Hospital
Edinburgh, Scotland, UK

Session 8
Day 3: Friday
8th February

Genetic and Environmental determinants of Paget's disease

Stuart H Ralston graduated in Medicine from Glasgow University in 1978 and underwent higher medical training in General Medicine and Rheumatology thereafter. He previously held the position of Professor of Medicine and Bone Metabolism at the University of Aberdeen and moved to Edinburgh University in 2005 to take up the Arthritis Research UK Chair of Rheumatology. Professor Ralston is an honorary consultant rheumatologist in NHS Lothian, and clinical lead for the osteoporosis service. He is editor in chief of Calcified Tissue International and also a member of the Commission for Human Medicines. Professor Ralston has researched widely on the molecular and genetic basis of bone and joint diseases and has a special interest in the pathogenesis and management of Paget's disease of bone.

Timothy Radstake

Department of Rheumatology
Radboud University
Nijmegen Medical Center
Nijmegen, The Netherlands

Session 8
Day 3: Friday
8th February

From Genes to fibrosis using the full spectrum of system biology

Professor Radstake MD, Ph.D. attended medical school at the Radboud University in Nijmegen the Netherlands where he graduated in 2000 with the judicium Cum Laude. He next performed his PhD on the genetic factors that determine the susceptibility to Rheumatoid arthritis and the role of dendritic cells in this conditions that he finished in 2003 (Cum Laude). Soon after that he developed his own research group focusing on the role of Toll-like receptors (TLR) and Fc gamma receptors in the regulation of dendritic cells function in rheumatoid arthritis. His group stood at the basis of the observation that TLR play a role in arthritis by dysregulating DC and macrophage function. Over the years his group investigated a broader spectrum of rheumatic conditions including psoriatic arthritis and systemic sclerosis. From 2007 – 2008 prof. Radstake did a post-doctoral fellowship on the department of rheumatology at the Boston University School of Medicine (prof. R. Lafyatis) where he currently still holds a position as associate professor on systemic sclerosis. From March 2012 he holds a full chair in translational immunology at the university medical Center Utrecht were his research team comprising out of 8 post-docs and 12 PhD students focuses on immune aspects of a broad spectrum of immune mediated diseases including SLE, Sjogrens syndrome, RA, PsA, systemic sclerosis and inflammatory myopathies. Together with prof. B. Prakken and Dr. M. Boes he is heading the clinical research line within the Laboratory of Translational Immunology encompassing ~ 80 fte working on translational immunology. His major aim is to understand how genetic alterations lead to epigenetic and immune dysregulation in immune mediated diseases and fibrosis. Ultimately, he is leading the effort to develop therapeutic targets to these conditions based on the genetic/epigenetic aberrances that are patient specific (patient tailored medicine).

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Tim Spector

Twin Research and Genetic Epidemiology Department
King's College London
London, UK

The Use of Twins in 'Omic Research

Tim Spector is a Professor of Genetic Epidemiology and Director of the TwinsUK Registry at Kings College, London. He trained in rheumatology and epidemiology, but moved into genetic epidemiology in 1992 when he founded the UK Twins Registry, with currently 11,000 twins and one of the richest collections of genotypic and phenotypic information on twins worldwide. He has published over 700 research articles and is an NIHR and ERC Senior Investigator. He is PI of many European and International genetic consortia and the author of several books on genetics and epigenetics, most recently "Identically Different".

Anne O'Garra
NIMR,
London, UK

A Systems Biology Approach to Study the Immune Response in Tuberculosis: Implications for Advancing Diagnosis and Monitoring of Drug Treatment

John Todd
University of Cambridge, UK

Genetically-validated intervention strategies in autoimmune diabetes

John Todd FRS, FMedSci, PhD is Professor of Medical Genetics at Cambridge University and Director of theJDRF/Wellcome Trust Diabetes and Inflammation Laboratory in the University's Cambridge Institute for Medical Research. Todd's researches type 1 diabetes genetics and disease mechanisms and clinical intervention, in collaboration with Linda Wicker, Chris Wallace and Frank Waldron-Lynch. He was a founder member of the consortia, the Wellcome Trust Case Control Consortium and the Type 1 Diabetes Genetics Consortium. Previously, Todd was a JDRF Career Development Fellow, Professor of Human Genetics at Oxford University and a Wellcome Trust Principal Research Fellow. He received his B.Sc. First class from Edinburgh University in Biological Sciences, and his Ph.D. from Cambridge University in Biochemistry. Post-doctoral training was undertaken at Cambridge and Stanford in genetics, molecular biology and immunology. He has over 420 publications, 288 of which were peer-reviewed with over 27,000 citations and H-index = 84, and has received several awards and honours for his research. He has trained so far 27 PhD students and many more research assistants and postdoctoral fellows. His main goal is to provide knowledge based on genetic aetiological findings that can inform in the prevention of autoimmunity in type 1 diabetes and in other immune-mediated diseases.

Reference:

Virgin, H.W. & Todd, J.A. (2011) Metagenomics and personalised medicine. Cell, 147, 44-56.

P1 Anita Grigoriadis: *The characterisation of triple-negative breast cancers by distinct types of genome instability*

Authors
Johnathan Watkins, Markus Mayrhofer, Anders Isaksson, Cheryl Gillett, Sarah Pinder, Sean Hooper, Rachel Brough, Jessica Frankum, Alan Ashworth, Christopher J. Lord, Anita Grigoriadis, Andrew Tutt

Affiliation
1 King's College London, London, UK
2. Uppsala University, Uppsala, Sweden
3. The Institute of Cancer Research, London, UK

Abstract text

Background
Triple Negative Breast Cancer (TNBC) is defined immunohistochemically by the absence of ER, PR and HER2. One approach to stratifying TNBC patients is to examine the degree and extent of genome instability (GI). Such an approach may enable prediction of response to drugs that capitalise on defects to DNA repair.

Method
Affymetrix Genome-wide SNP 6.0 profiles were generated and allele-specific copy-number patterns established using Tumor Aberration Prediction Suite for 111 TNBCs and a panel of 33 breast cancer cell-lines. We calculated three scores for each sample, which define different types of chromosomal GI: allelic-imbalanced copy number aberrations (aiCNA), allelic-balanced copy-number aberrations (abCNA) and copy number-neutral loss of heterozygosity (cnLoH). These scores were compared to the clinico-pathological features of the tumour panel, as well as RNAi functional viability profiles and drug-sensitivity data for the cell line panel.

Results
Scoring of each tumour by genomic scar revealed a varying degree of cnLoH, aiCNA and abCNA across the TNBC cohort. Clustering by GI scar score revealed two robust groups: one with high levels of cnLoH relative to aiCNA, and one with markedly higher aiCNA. Tumour necrosis was found to be significantly associated with aiCNA but not with cnLoH. Breast cancer cell line models shared the GI score spectra and exhibited differing association with drug and siRNA sensitivity.

Conclusion
TNBCs can be characterised by their level of aiCNA and cnLoH, each of which may be a result of distinct, predominant GI mechanisms that relate to differential clinico-pathological phenotypes, drug response and sensitivity to gene inactivation.

P2 Emmanuel Karouzakis: *Epigenome analysis of rheumatoid arthritis synovial fibroblasts revealed TBX-5 as a novel transcription factor in chemokine regulation.*

Authors
Emmanuel Karouzakis, Michelle Trenkmann, Renate E. Gay, Beat A. Michel, Steffen Gay, Michel Neidhart

Affiliation
Center of Experimental Rheumatology and Zurich Center of Integrative Human Physiology (ZIHP), University Hospital Zurich, Switzerland

Abstract text
Although the present workshop is focused on the impact of genetic variations in rheumatic diseases. It appears important to explore the functional role of these variations in regulating epigenetic modifications and gene expression. Here, we report on the modulation of gene expression, focusing on DNA methylation and histone modifications which are imprinted in the chromatin and constitute the epigenetic code. In this study, we analyzed the methylation status of human promoters in rheumatoid arthritis synovial fibroblasts. Differentially methylated genes between RASFs versus OASFs were identified by methylation immunoprecipitation and hybridization of human promoter tiling arrays. The methylation status was confirmed with pyrosequencing assays. The gene and protein expression of differential methylated genes were evaluated with Real time PCR, Western blot and immunohistochemistry. Chromatin immunoprecipitations assays were used to analyze the acetylation and methylation of histone proteins. The identification of transcription factor specific targets was analysed with microarray and luciferase assays. In RA synovium and RASF, TBX5 was found to be less methylated than in OASF. The demethylation of TBX5 promoter in RASF and RA synovium induced higher TBX5 expression than in OASF and OA synovium. In RA synovium, TBX5 was mostly localized in the synovial lining. In addition, the TBX5 locus was found to be enriched in RASF with open chromatin marks such as H3K4 trimethylation and histone acetylation. Overexpression of TBX5 in OASF revealed 640 genes commonly upregulated from 1.2 to 3-fold. Analysis of these genes by DAVID bioinformatics tool identified that the chemokines IL8, CXCL12 and CCL20 were novel targets of TBX5 in OASF.TBX5 may be an important regulator of chemotaxis associated with the ability of RASF to attract inflammatory cells to the synovium.

P3 Ian Scott: *Predicting The Risk Of Rheumatoid Arthritis And Its Age Of Onset Through Modelling Genetic Risk Variants With Smoking*

Authors
Ian C. Scott, Seth Seegobin, Sophia Steer, Rachael Tan, Paola Forabosco, Anne Hinks, Stephen Eyre, Ann W. Morgan, Anthony G. Wilson, Lynne J. Hocking, Paul B. Wordsworth, Anne Barton, Jane Worthington, Andrew P. Cope, Cathryn M. Lewis

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Abstract text

Objectives

The improved characterisation of Rheumatoid Arthritis' (RA's) risk factors suggests they could be combined to identify individuals at increased disease risks in whom preventive strategies may be evaluated. The large risks provided by functionally important HLA-DRB1 alleles suggest prediction modelling may have clinical utility in RA, despite limited success in other complex diseases. We aimed to develop a prediction model capable of generating clinically relevant predictive data and to determine if it better identified young onset RA (YORA)/severe RA phenotypes.

Methods

Our novel modelling approach combines odds ratios for 15 HLA-DRB1 alleles, 31 single nucleotide polymorphisms (SNPs) and smoking status to determine risk using confidence interval-based categorisation and computer simulation. We developed multiple models (SNP, HLA, HLA-SNP, HLA-smoking, HLA-SNP-smoking models) to evaluate different factors impacts on prediction. Each model's ability to discriminate antibodies to citrullinated protein antigens (ACPA)-positive RA from controls was evaluated in two cohorts: Wellcome Trust Case Control Consortium (WTCCC: 1,061 cases; 1,647 controls); UK RA Genetics Group Consortium (UKRAGG: 1,508 cases; 1,500 controls).

Results

HLA and smoking provided most prediction. SNPs had minimal predictive utility. The highest area under the curve (HLA-SNP-smoking model) was 0.857 (95% CI 0.804-0.910) indicating good discrimination. Only a minority were at substantially elevated risks: 3.75-7.53% cases had lifetime RA risks >22% using this model. The HLA model better identified those at risk of YORA (WTCCC: $P<0.0001$; UKRAGG: $P=0.0004$). Ever-smoking inversely associated with YORA in UKRAGG ($P=0.0009$).

Conclusions

Our modelling demonstrates that combined gene-environment risk factor information provides informative RA prediction. Most prediction is from the HLA region and smoking, which play important functional roles in citrullinated peptide neoepitope generation/self-tolerance breakdown. Furthermore HLA and never-smoking status better predict YORA. As only a minority have substantially elevated risks our modelling requires evaluation in a priori high-risk groups to determine its clinical relevance.

P4 Klementy Schcetynsky: *Gene-gene interaction and RNA splicing profiles of MAP2K4 gene in rheumatoid arthritis*

Authors

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Abstract text

Background and objectives

MAP2K4 encodes a mitogen activated protein kinase kinase 4 (MKK4), important for optimal activation of JNK1-3 and p38 – the two members of the MAP kinase family.

In this study we explore the epistatic relations between MAP2K4 locus and two major known genetic risk factors for autoantibody positive rheumatoid arthritis (RA) -- HLA-DRB1 shared epitope (SE) alleles and PTPN22rs rs2476601. We also address the expression of MAP2K4 splicing forms and its association with known SE and PTPN22 genotypes and autoantibody profile of the disease.

Methods

The genotypes from 1985 patients with RA and 2252 matched healthy controls from the Swedish EIRA study population and from 863 RA cases and 1181 controls from the NARAC were used in the study. The interaction analysis was performed on 22 SNPs from the MAP2K4 locus, HLA-DRB1 shared epitope alleles and rs2476601 from PTPN22 by calculation the attributable proportion due to interaction (AP).

We studied transcript diversity of MAP2K4 and investigated relative expression of MAP2K4 forms in peripheral mononuclear cells for 44 RA patients and 44 controls of Caucasian ancestry. These results were analyzed against available genotypic and phenotypic data.

Results

We found MAP2K4 rs10468473 in statistical interaction with SE, and PTPN22 rs247660 in ACPA-positive RA in two independent cohorts.

We detected a novel "skipped exon" type splice variant of MAP2K4 in our study material. The MAP2K4 splice forms were differentially expressed in peripheral blood material from 88 RA cases and controls. Within the group of RA patients, a correlation was observed between MAP2K4 variants expression and phenotypic data for ACPA, rheumatoid factor, and SE.

Conclusion

Our data suggest epistatic relationships between MAP2K4, PTPN22 and HLA-DRB1 in development of ACPA positive rheumatoid arthritis. Possible mechanism of action may involve a change in expression of alternatively spliced MAP2K4 mRNAs in RA patients.

P5 Jonathan Watkins: *The characterisation of triple-negative breast cancers by distinct types of genome instability*

Authors
Johnathan Watkins, Markus Mayrhofer, Anders Isaksson, Cheryl Gillett, Sarah Pinder, Sean Hooper, Rachel Brough, Jessica Frankum, Alan Ashworth, Christopher J. Lord, Anita Grigoriadis, Andrew Tutt

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3. The Institute of Cancer Research, London, UK

Abstract text

Background
Triple Negative Breast Cancer (TNBC) is defined immunohistochemically by the absence of ER, PR and HER2. One approach to stratifying TNBC patients is to examine the degree and extent of genome instability (GI). Such an approach may enable prediction of response to drugs that capitalise on defects to DNA repair.

Method
Affymetrix Genome-wide SNP 6.0 profiles were generated and allele-specific copy-number patterns established using Tumor Aberration Prediction Suite for 111 TNBCs and a panel of 33 breast cancer cell-lines. We calculated three scores for each sample, which define different types of chromosomal GI: allelic-imbalanced copy number aberrations (aiCNA), allelic-balanced copy-number aberrations (abCNA) and copy number-neutral loss of heterozygosity (cnLoH). These scores were compared to the clinico-pathological features of the tumour panel, as well as RNAi functional viability profiles and drug-sensitivity data for the cell line panel.

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Conclusion
TNBCs can be characterised by their level of aiCNA and cnLoH, each of which may be a result of distinct, predominant GI mechanisms that relate to differential clinico-pathological phenotypes, drug response and sensitivity to gene inactivation.

P6 Anna Zampetaki: *Circulating MicroRNAs as Novel Biomarkers for Platelet Activation*

Authors
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Abstract text

Rationale
MicroRNA biomarkers are attracting considerable interest. Effects of medication, however, have not been investigated thus far.

Objective
To analyse changes in plasma microRNAs in response to anti-platelet therapy.

Methods and Results
Profiling for 377 microRNAs was performed in platelets, platelet microparticles, platelet-rich plasma, platelet-poor plasma and serum. Platelet-rich plasma showed markedly higher levels of microRNAs than serum and platelet-poor plasma. Few abundant platelet microRNAs, such as miR-24, miR-197, miR-191, and miR-223, were also increased in serum compared to platelet-poor plasma. In contrast, anti-platelet therapy significantly reduced microRNA levels. Using custom-made qPCR plates, 92 microRNAs were assessed in a dose-escalation study in healthy volunteers at four different time points: at baseline without therapy, at 1 week with 10mg prasugrel, at 2 weeks with 10mg prasugrel+75mg aspirin and at 3 weeks with 10mg prasugrel+300mg aspirin. Findings in healthy volunteers were confirmed by individual TaqMan qPCR assays (n=9). Validation was performed in an independent cohort of patients with symptomatic atherosclerosis (n=33) who received low dose aspirin at baseline. Plasma levels of platelet microRNAs, such as miR-223, miR-191 and others, i.e. miR-126 and miR-150, decreased upon further platelet inhibition.

Conclusions
Our study demonstrated a substantial platelet contribution to the circulating microRNA pool and identified microRNAs responsive to anti-platelet therapy. It also highlights that anti-platelet therapy and preparation of blood samples could be confounding factors in case-control studies relating plasma microRNAs to cardiovascular disease

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