Expression profiling of 4p terminal deletions suggest that telomere position effects may contribute to disease phenotypes

Femke D. L. C. Hannes; Martin Poot; Jean-Pierre Fyrs; Koen Devriendt; Joris Vermeesch.
Centre for Human-Genetics, University Hospital, Catholic University of Leuven, Belgium

Email: joris.vermeesch@uz.kuleuven.be;

Alteration in gene expression as a result of genetic imbalances are involved in a wide range of human diseases. However, little is known about altered gene expression of genes flanking the aberration in human disease. Wolf-Hirschhorn syndrome (WHS) is a well defined clinical entity and characterised by terminal or interstitial deletions of chromosome 4p. The description of interstitial as well as terminal deletions makes WHS an ideal model to investigate two types of position effects a) Telomere Position Effects or TPE, were the presence of a telomere influences the nearby gene-expression and b) position effects caused by interstitial deletions. EBV cell lines were established from 7 WHS patients, 3 with interstitial and 4 with telomeric submicroscopic deletions. Expression profiles of 56 genes using Real-Time quantitative PCR were determined in these 7 patients and 10 normal control cell lines. 30 of those genes were expressed in EBV cells and expression was quantified. In 3 patients with interstitial and 3 patients with terminal deletions all genes showed dosage specific expression. Genes within the deleted region are expressed at half the dosage as compared to genes present in two copies. In one patient, two genes next to the putative breakpoint and three genes located at 1 Mb from the subtelomere were shown to be downregulated. These results suggest that telomeric and interstitial position effects may not be very common. However, the downregulated expression of subtelomeric genes in one patient may indicate that TPE can occur in human broken chromosomes.

Gene sets enrichment analysis of chromosomal regions

Kathleen de Preter; Roland Barriot; Frank Speleman; Jo Vandesompele; Yves Moreau.
SymBioSys / ESAT-SCD, Leuven, Belgium

Email: roland.barriot@esat.kuleuven.be

The search for feature enrichment is a widely used method to characterize a set of genes. While several tools have been designed for nominal features such as Gene Ontology annotations or KEGG Pathways, very little has been proposed to tackle numerical features such as the chromosomal positions of genes. For instance, microarray studies typically generate gene lists that are differentially expressed in the sample subgroups under investigation, and when studying diseases caused by genome alterations, it is of great interest to delineate the chromosomal regions that are significantly enriched in these lists. In this article, we present a positional gene enrichment analysis method (PGE) for the identification of chromosomal regions that are significantly enriched in a given set of genes. The strength of our method relies on an original query optimization approach that allows to virtually consider all the possible chromosomal regions for enrichment, and on the multiple testing correction which discriminates truly enriched regions versus those that can occur by chance. We have developed a Web tool implementing this method applied to the human genome (http://www.esat.kuleuven.be/~bioiuser/pge). For validation, we used PGE on published lists of differentially expressed genes observed in B-cell chronic lymphocytic leukemia, neuroblastoma tumors and tissues of Down syndrome patients. These analyses showed significant overrepresentation of known aberrant chromosomal regions.

To be announced
HERITABILITY ESTIMATES OF ANTI-MYCOBACTERIAL IMMUNE RESPONSES

Caroline J. Gallant; Aurelie Cobat; Alexandre Alcaïs; Gillian F. Black; Kim Stanley; Willem A. Hanekom; Jane Hughes; Brian Eley; Nulda Beyers; Paul Helden van; Laurent Abel; Eileen G. Hoal; Erwin Schurr.
Centre for the Study of Host Resistance, McGill University, McGill University, Canada

Numerous studies have identified a strong genetic component contributing to tuberculosis susceptibility. However, little is known about how gene variants affect host immune responses to Mycobacterium tuberculosis. Identification of these genes is essential to identify critical pathways of tuberculosis pathogenesis. Here, we report on genetic studies of three immune phenotypes that capture variable aspects of human responses to mycobacteria. A total of 155 multiplex families, including 522 children, were enrolled in Cape Town, South Africa, from an area with one of the highest reported tuberculosis incidence rates globally. The enrolment criteria aimed to maximize the number of children from large families with a high probability of previous mycobacterial exposure. Three types of immune phenotypes were determined on all enrolled children: i) Mantoux skin test reactivity, ii) mycobacteria antigen-induced IFNγ and TNFα production by blood lymphocytes and iii) frequency of mycobacteria-induced, IFNγ producing CD4+ T cells in peripheral blood. Stimulating antigens used were: live BCG, M. tuberculosis purified protein derivative (PPD), and M. tuberculosis secreted protein ESAT-6. Over 55% of children displayed evidence of mycobacterial exposure using Mantoux skin test reactivity (>10 mm) as a measure. With the exception of mycobacteria-induced TNFα production, considerable correlations are observed between responses to the antigens tested in the blood based assays and the Mantoux skin test reaction. Heritability was calculated for immune phenotypes i) and ii) and an estimate as high as 80% was found for BCG-triggered TNFα production. Heritability for IFNγ production in response to ESAT6, PPD and BCG was estimated to be 60%, 55% and 45% respectively. Mantoux heritability was estimated to be 22%. Heritability for the frequency of mycobacteria-induced, IFNγ producing CD4+ T cells will also be calculated. Our estimates are in-line with published studies. Given these high estimates of trait heritability, simulation studies show that we have excellent power to detect the underlying gene variants employing forward genetics approaches.

SEARCHING FOR A COMMON GENETIC FACTOR FOR BEHÇET’S DISEASE AND ANKYLOSING SPONDYLITIS

Joana M. Xavier; Tiago Krug; Filipe Barcelos; Benedita Vasconcelos Fonseca; Ana Teixeira; José Vaz Patto; Sofia Almeida Oliveira.
Instituto Gulbenkian de Ciencia, Portugal

Introduction: Behçet’s disease (BD) is a chronic inflammatory disorder, classified as a vasculitis, that may involve several organs, such as skin, mucocutaneous membranes (oral and genital aphthae), eyes, joints, lungs, gastrointestinal and central nervous systems. Ankylosing spondylitis (AS) is a chronic inflammatory condition of the axial skeleton, mainly affecting the sacroiliac joint, spine and enthesis but also frequently involving the eyes, heart, lungs and gastrointestinal system. These disorders are thought to be multifactorial, with several genetic and environmental factors implicated in their etiology. The only genes that have been consistently associated worldwide with these diseases belong to the Major Histocompatibility Complex (MHC). HLA-B*51 explains up to 19% of the BD risk, and HLA-B*27 has an attributable risk of 20-40% for AS. The previously reported co-occurrence of BD and AS in some patients, their clinical similarities and their aggregation in a Portuguese family led us to hypothesize that BD and AS may share common genetic factors and to search for such factors in an extended multicase family.

Methods & Materials: We performed high-density genotyping (using the Affymetrix gene Chip 250K StyI platform) in a family with 22 individuals, 3 of which are affected with BD and 4 affected by AS. Copy number variation (CNV) and loss of heterozygosity (LOH) were assessed using the Affymetrix CNAT4.0 software and non-parametric linkage analysis carried out using Merlin. Results: There was no indication of LOH and no overall differences in CNV were found. A joint linkage analysis for BD and AS found strong evidence for linkage (LOD score of 3.59) on chromosome 8p21.3-22. A fine haplotyping analysis confined the disease locus to a 3.6 megabases region containing 24 genes.

Conclusions: This is the first study identifying a non-MHC genetic locus for both BD and AS. A mutation search in the genes under the linkage peak is underway. The discovery of new genetic risk factors for these
disorders constitutes a clear path for a better understanding of their etiopathology and for the development of novel and more specific therapeutic interventions.

**IL.16 12.15 – 13.00**

**COLORECTAL CANCER EPIGENETICS: FROM BIOLOGY TO BIOMARKER**

Manon van Engeland,
Dept. of Pathology, GROW- School for Oncology & Developmental Biology, Maastricht University

Email: m.vanengeland@path.unimaas.nl

*Introduction:* Aberrant promoter methylation is involved in transcriptional silencing of tumour suppressor- and DNA repair genes in colorectal cancer and has been proposed as biomarker for early detection of this disease. *Methods & Materials:* In order to identify novel tumor suppressor genes and sensitive and specific methylation markers for detection of colorectal cancer we performed genome-wide epigenetic screens. Novel methylation markers are validated in large, well characterised series of tissue, stool and blood of colorectal cancer cases and controls using MSP, bisulfite sequencing and RT-PCR. Tumor suppressor function of novel candidate markers is analysed using colony formation, cell proliferation, migration, invasion and anchorage-independent growth assays. Comparison of the efficacy and cost-effectiveness of methylation markers against the current gold standards colonoscopy and FOBT is currently ongoing. *Results:* Here I present the identification of novel candidate colorectal cancer suppressor genes and candidate methylation markers. *Conclusions:* These epigenetic screens provide 1) novel insight in the tumor biology of colorectal cancer and 2) novel methylation markers for the early detection of colorectal cancer.

**O23 13.00 – 13.15**

**THE CONSEQUENCES OF CHROMOSOME 1Q GAINS ON GENE EXPRESSION**

Vivien Boldt; Artur Muradyan; Anne Steininger; Ines Muller; Hannelore Madle; Reinhard Ullmann.
Max Planck Institute for Molecular Genetics, Berlin, Germany

Email: boldt@molgen.mpg.de

*Introduction:* Recurrent DNA copy number changes have gained increasing attention as means to identify genes important in tumorogenesis as well as markers assisting differential diagnosis and therapeutic decisions. The basic assumption underlying this concept, however, is that a certain chromosome aberration always entails the same biological consequences. Yet, this scenario is rather unlikely, given the individual epigenetic and genetic background in each tumour, resulting in the abundance or depletion of different sets of transcription factors and other regulatory elements. In this study we set out to investigate the variability of gene expression signatures within a cohort of eight cell lines sharing a gain of whole chromosome arm 1q and compared our results to a group of seven cell lines lacking these aberrations and two cell lines showing a loss of this region. *Methods & Materials:* For all 17 cell lines, DNA copy number changes were detected using a submegabase resolution BAC array and gene expression analysis was performed on the 44k oligoarray from Agilent. 1858 probes of this platform mapped to chromosome 1q. All cell lines were classified in one out of three groups (1. with 1q gain; 2. balanced status; 3. 1q loss). *Results:* Based on a pairwise comparison between the averages of the three groups, 189 genes were constantly up- and 23 downregulated in group 1. All cell lines were classified in one out of three groups (1. with 1q gain; 2. balanced status; 3. 1q loss). *Results:* Based on a pairwise comparison between the averages of the three groups, 189 genes were constantly up- and 23 downregulated in group 1. For 1396 genes, their average expression level within each of the three groups showed no obvious influence by DNA copy number change. *Conclusions:* Here we show that only a small minority of genes are regularly overexpressed in response to 1q gains, with some genes even showing the opposite effect. We will further discuss the presence of genes that apparently do not tolerate any dosage alteration and report on our current efforts to supplement our data with ChIP on Chip experiments to investigate epigenetic modifications and selected transcription factors.

**13.15 – 14.30**

**CLOSING REMARKS AND LUNCH**