The Human Genome Organisation

CERTIFICATE OF ATTENDANCE

THIS CERTIFICATE CONFIRMS THAT

Dr Mohammad Mahdi Ghahramani Seno

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participated in the

12th HUGO HUMAN GENOME MEETING

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Catherine J Pole

Catherine J Pole
Executive Director
HUGO

underlying interindividual variability of mRNA levels have been demonstrated in immortalized human cell lines, but variants underlying induced gene expression in primary cells as described here have not been studied to date. Herein, we used primary human osteoblasts derived from unrelated donors undergoing total hip replacement to examine natural variation in induced gene expression. Three independent cell cultures from each individual were used and included in subsequent analyses. We have now progressed to identification of interindividually variable induction phenotypes upon multiple trophic stimuli. For example, after stimulation with bone morphogenic protein 2 (BMP2) - a protein involved in bone deviopment and osteoblast differentiation - multiple signaling pathways known to stimulate osteoblastogenesis were rapidly activated, which was assessed by expression profiling (Affymetrix HGU133). In order to study interindividual variability of the induced gene expression, quantitative RT-PCR on BMP2 responded genes within the biochemical pathways was performed. The genes studied included SMAD6, SMAD7, CXCL2, ID1, ID2, HES1, FGF18, and DLX2. In seven out of the eight selected genes, the squared difference in expression levels between individuals was higher than the squared difference of expression levels within. The fold changes in terms of difference between samples vs. within samples of the seven genes ranged from 1.4 to 6.9 with a median fold change of 2.6. These findings may indicate that the variation of induced gene expression in human primary osteblasts is genetically determined. The next steps will be to map variation in human osteoblast responses by candidate gene and whole genome association studies. This work is supported by Genome Quebec and Genome Canada

WORKSHOP ABSTRACT NO: 5
Functional impact of regulatory polymorphisms (rSNP) in G1/S cell cycle checkpoint genes

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G1/S transition in the cell cycle is a finely regulated biological process. Depending on the context, growth-control genes present in the G1/S checkpoint can stop the cell cycle progression and activate survival pathway in the cell. Then DNA repair process or cell death by apoptosis is initiated. Dysregulation of G1/S checkpoint genes is frequently observed in complex disease, particularly like cancer. Indeed gene encoding components of cell cycle processes are frequently mutated in human cancers. We hypothesize that functional polymorphisms located in the regulatory region of candidates genes could lead to variable level of transcript and thus predisposing the individuals carrying these genetic variants to cancer. In this report we assessed the functional impact of rSNPs located in the proximal promoter of 18 candidates genes encoding components of G1/S checkpoint by combining in silico analysis and in vitro functional assays. We identified 150 rSNPs including 123 with predicted impact on putative transcription factor binding sites. This information was used to construct promoter haplotypes (rHAP). Following the subcloning of the major promoter haplotypes into a luciferase gene reporter vector (pGL3b), transient transfection assays were performed in 3 cell lines (Hela, Jeg3 and HepG2). We found that at least 11 rHAPs associated with TGFB1, TFDP1, CDKN1A, CDKN1B, CDKN2A and CDKN2B significantly influenced transcriptional activity in an allele-specific manner. Further validation by electrophoresis mobility shift assays (EMSA) to detect differential DNA-protein bindings is being done. Although, the biological significance of these observations still remain to be demonstrated, the expected variability of expression levels in key cell cycle components might influence individual's risk of cancer.

WORKSHOP ABSTRACT NO: 6
Novel Regulatory Regions in the Interleukin 6 Gene and Implications for Disease/Gene Association Studies

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Interleukin-6 (IL-6, encoded by gene IL6) is an important pleiotropic cytokine that is regulated at the transcriptional level. To date, most work on its regulation has focused on a 1.2kb region 5' from the start of transcription. We have identified the -174 G allele of the IL6 gene as a susceptibility loci in systemic JIA . This variant was also shown to influence IL6 transcription and serum levels. However, our recent investigation of the functionality of -174 G/C, with additional polymorphisms in the IL6 promoter, indicated a more complex regulatory haplotype extending further upstream of the previously analysed promoter region. The involvement of a much larger upstream region in the regulation of IL6 is described in this report. Comparative genomics analysis has identified a 120kb region which contains blocks of sequence conservation between human and rodent genomes. Additionally, the 15kb region proximal to the start of transcription contains ten highly homologous sequence blocks of between 100-250bp. By means of a reporter gene assay, a novel 105bp transcriptionally active region, located 5kb upstream from the start of transcription, was identified. Further evidence that this sequence is involved in the regulation of IL6 is indicated by the binding of protein(s) to this region using electrophoretic mobility shift assays. To determine if any common variation was present in this region it was sequenced using the DNA from 24 healthy individuals. No variation in sequence was identified. Both the -174 C and G alleles have been associated with numerous autoimmune and inflammatory diseases. These associations have been inconsistent between studies, so it is possible that these differences can be explained by the presence of additional nucleotide variation in other regions important for transcriptional regulation. Our study has identified a region important in the regulation of IL6 transcription, but no common polymorphisms were identified in this region. Work is currently focusing on identifying polymorphisms, within the 120kb conserved upstream region of IL6, that could explain the anomalies in the associations between IL6 -174 genotype and IL-6 levels. In summary, the results of this study suggest that the regulation of IL6 expression involves a much larger upstream region than previously examined, and the control of IL6 transcription is likely to be regulated by a complex mechanism of modular cis-regulatory elements.

WORKSHOP ABSTRACT NO: 7
PTCHD3 gene: a copy number variable gene in humans

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Genomic copy number variation (CNV) in the human population is a well-documented source of genetic variation covering at least 10-12% of the genome. Here we identify a new CNV of ~90kb at chromosome 10p12.1 encompassing the PTCHD3 gene. This CNV was originally detected as deletion in a number of patients diagnosed with Autism Spectrum Disorder. However, further investigations demonstrate that the CNV is likely not disease-associated but instead a rare variant mainly in Caucasian populations appearing at frequency of ~0.5-1%. The deletion is mainly heterozygous, although homozygous deletions at this locus appear to be tolerated without obvious phenotypic effect. The presence of apparently healthy individuals null for PTCHD3 suggests it may be a non-essential human gene, a somewhat surprising observation given its high evolutionary conservation amongst divergent species.

The human PTCHD3 gene is composed of four exons covering ~16kb of genomic DNA with an mRNA transcript of ~2.4kb in size. The protein product is 767 amino acids in length with a 595 residue patched domain that is highly conserved in vertebrates. Based on its amino acid composition and similarity comparisons, it is modelled as a transmembrane protein with hedgehog receptor activity. Our work reveals the gene