2013

# Book of Abstracts
2013

## Nobel Day Festivities
10th of December 2013


Traditionally, on 10th of December, the anniversary of Alfred Nobel's death, is awarded the Nobel Prize in Physiology or Medicine. School of Health and Medical Sciences shows attention to this day by organizing own research activities and festivities.

School of Health and Medical Sciences
Örebro University
10th of December 2013

**Program Committee**:
Professors; Nikolaos Venizelos, Allan Sirsjö, Olle Ljungqvist,
Ulrica Nilsson, Magnus Grenegård, Charli Eriksson, Christer Ericsson

Book of abstracts, Nobel Day's Festivities 10th of December 2013

## Preface

The”**Nobel Day Festivities”** were established 2009 by Biomedicine, Department of Clinical Medicine, School of Health and Medical Sciences at Örebro University, and is organized traditionally every year in order to notice the anniversary of Alfred Nobel's death (10th of December) with scientific activities including poster presentations and selected oral presentations by doctoral students, which are documented in this *“Book of abstracts”*. Nobel day's activities are open and scheduled so that all students and personnel can attend the scientific activities. We warmly welcome you to enjoy all the good science that will be presented at Nobel Day.

The Organizers

Nikolaos Venizelos, Professor em
Allan Sirsjö, Professor
Olle Ljungqvist, Professor
Ulrica Nilsson, Professor
Magnus Grenegård, Professor
Charli Eriksson, Professor
Christer Ericsson, Professor

# Exploring the concept of optimal functionality in old age (*)

**Samal Algilani[1]**, Lina Östlund-Lagerström[1,2]**, Annica Kihlgren[1], Robert J. Brummer[1,2], Ida Schoultz[1,2]**

*(**Both authors contributed equally to this work)*

[1]Nutrition and Physical Activity Research Centre, [2]Nutrition Gut Brain Interactions Research Centre, School of Health and Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden.

**Objective:** Ageing is characterized by loss of function and represents a perspective that put the negative aspects of ageing in focus. We wish to shift the focus from loss of function to maintaining good health and personal satisfaction. Thus, the aim of this paper is to explore the concept of optimal functionality in old age from the older adult´s own perspective.

**Methods:** In order to explore the concept of optimal functionality, we undertook a scoping review and searched two electronic databases (CINAHL and PubMed) from January 2002 – July 2013 for scientific studies, using the key search term *personal satisfaction*. In total 25 scientific studies were analyzed.

**Results:** Optimal functionality includes three main themes: self-related factors, body-related factors, and external factors. Further, only six of the 25 included articles were found to apply a qualitative methodology.

**Conclusion:** Our results can be seen as a first step towards defining optimal functionality. We also conclude that there is a lack of knowledge regarding studies based on qualitative data. Thus, we call for further qualitative studies on the subject emphasizing the older adult's own opinion on what promotes optimal functionality.

*(*) This paper is submitted in J Multidiscip Healthc.*

# Cumulative effects of TNF and hypoxia increase VHL expression and disrupt hypoxia-angiogenesis signaling pathway in C2C12 myocytes

**Vladimir T. Basic[1], Annette Jacobsen[1,2], Allan Sirsjö[1] and Samy M. Abdel-Halim[3]**

[1]Department of Clinical Medicine, Örebro University, Örebro, Sweden
[2]School of Biomedical Sciences, Charles Sturt University, WaggaWagga, Australia
[3]Division of Respiratory Medicine and Allergology, Department of Clinical Sciences, Danderyd Hospital, Stockholm, Sweden

**Objectives:** Elevated circulatory levels of pro-inflammatory cytokines are believed to play an important causal role in the development of cachexia and skeletal muscle atrophy in different chronic inflammatory diseases including cancer, AIDS, congestive heart failure, and COPD. Whether there is a connection between systemic inflammation and impaired skeletal muscle capillarization observed in these conditions is currently not known. This study was designed to examine effects of pro-inflammatory cytokine TNF on hypoxia angiogenesis signaling cascade and skeletal muscles capillarization using an *in-vitro* C2C12 myocytes model.

**Methods:** Fully differentiated C2C12 were stimulated with TNF and maintained in normal oxygen conditions as well as exposed to hypoxic conditions. Expression of putative elements of hypoxia-angiogenesis signaling cascade was studied using qPCR, western blot and immunocytochemistry. Increase in total protein ubiquitination was measured using western blot. Additionally, localization of VHL and associated members of UPS cascade in murine TA has been assessed using immunohistochemistry.

**Results:** TNF increases protein expression of VHL, PHD2 and Ube2D1 in C2C12 myocytes, while Ube1 was not differentially expressed. In contrast, expression of HIF1-α and its transcriptional targets: including VEGFA, VEGFB, Glut1 and GAPDH was reduced. Hypoxia increases expression of VHL transcript and further elevates VHL protein expression in C2C12 after TNF stimulation. Additionally, blunted angiogenic response to hypoxia challenge has been observed in C2C12 after TNF stimulation.

**Conclusions:** In summary, TNF disrupted hypoxia-angiogenesis signaling cascade in C2C12 myocytes via stabilization of VHL and activation of UPS. Cumulative effect of hypoxia exposure and TNF stimulation resulted in robust increase in VHL expression and blunted angiogenic response of C2C12 to hypoxia insult. Taken together our data suggest the possibility that systemic inflammation and elevated circulatory TNF adversely affect angiogenic and glycolytic capacity of peripheral musculature in chronic inflammatory diseases, further contributing to the development of skeletal muscle dysfunction and disability in these conditions.

**References:**

1. *Basic VT, Tadele E, Elmabsout AA, Yao H, Rahman I, et al. (2012) Exposure to cigarette smoke induces overexpression of von Hippel-Lindau tumor suppressor in mouse skeletal muscle. Am J Physiol Lung Cell Mol Physiol. United States. pp. L519-527.*

# Ubiquitin specific protease 19 is up-regulated in skeletal muscles of murine model of COPD/emphysema. Possible connection to skeletal muscle atrophy

**Vladimir T. Basic[1], Annette Jacobsen[1,2]**

[1]Department of Clinical Medicine, Örebro University, Örebro, Sweden;
[2]School of Biomedical Sciences, Charles Sturt University, WaggaWagga, Australia

**Objectives:** Cigarette smoking (CS) represents the main causative agent underlying development and progress of COPD. Recently, involvement of CS in the pathogenesis of COPD-associated muscle abnormalities is becoming increasingly evident. Here, we report that chronic cigarette smoke exposure increases expression of Usp19 and induces ER stress in skeletal muscles of murine COPD/emphysema model. Usp19 is a well described promoter of muscle catabolism as well as regulator of cellular response to diverse stress stimuli such as ER stress and hypoxia.

**Methods:** 129 SvJ mice were exposed to CS for 6 months, specimens collected from gastrocnemius muscle and the expression levels of Usp19 as well as pivotal mediators of ER stress response have been studied. In addition, promoter sequence analysis of murine Usp19 has been performed. Furthermore C2C12 myocytes were stimulated with different inducers of ER tress including hypoxia, TNF and tunicamycin and Usp19 expression was assessed using qPCR, western blot and immunocytochemistry.

**Results:** 6 months of CS exposure elevated mRNA and protein levels of Usp19 and increased expression of activated Caspase 12 and Caspase 3 in gastrocnemius muscle of 129 SvJ mice. Analysis of Usp19 promoter sequence revealed putative binding sites for stress response transcription factors such as HSF, STRE1 and AML1-α. In accordance, pharmacological inducer of ER stress, tunicamycin elevated Usp19 mRNA levels in C2C12 myocytes. Additionally, in vitro analysis demonstrated that Usp19 is not a hypoxia regulated gene while TNF stabilized Usp19 protein but inhibited Usp19 transcription in a dose and time dependent manner.

**Conclusions:** In summary, our data demonstrates elevated expression of Usp19 as well as presence of chronic ER stress in skeletal muscles of CS-exposed 129 SvJ mice. Furthermore, in-vitro studies demonstrated that elevation of Usp19 expression is a part of ER stress response in C2C12 myocytes. This might provide further insight into molecular mechanism underlying development and progression of skeletal muscle abnormalities in response to CS.

**References:**

*1. Liu Q, Xu WG, Luo Y, Han FF, Yao XH, et al. (2011) Cigarette smoke-induced skeletal muscle atrophy is associated with up-regulation of USP-19 via p38 and ERK MAPKs. J Cell Biochem 112: 2307-2316.*

*2. Basic VT, Tadele E, Elmabsout AA, Yao H, Rahman I, et al. (2012) Exposure to cigarette smoke induces overexpression of von Hippel-Lindau tumor suppressor in mouse skeletal muscle. Am J Physiol Lung Cell Mol Physiol. United States. pp. L519-527.*

# Chronic cigarette smoke exposure impairs skeletal muscle regenerative capacity in murine COPD/emphysema model

**Vladimir T. Basic[1], Elsa Tadele[1], Annette Jacobsen[1,2], Allan Sirsjö[1] and Samy M. Abdel-Halim[3]**

[1]Department of Clinical Medicine, Örebro University, Örebro, Sweden;
[2]School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, Australia
[3]Division of Respiratory Medicine and Allergology, Department of Clinical Sciences, Danderyd Hospital, Stockholm, Sweden

**Background:** Cigarette smoke (CS) is a well established risk factor in the development of irreversible airflow limitation in COPD. In contrast, the extent to which CS exposure contributes to development of skeletal muscle dysfunction and wasting remains largely unknown. Decline in skeletal muscle regenerative capacity has been previously reported in COPD patients.

**Methods:** To investigate the effects of chronic CS exposure on skeletal muscle regenerative capacity, 129/SvJ mice were exposed to CS for 6 months. The expression levels of myogenin, Jarid2, Znf496, Notch1, Pax7, Fgf1 and Myh3 which are known to regulate skeletal muscle myogenesis, were studied. Additionally, number of fibers with central nuclei, myonuclei number and mean fiber cross-sectional area were assessed.

**Results:** Compared to controls, skeletal muscles from CS-exposed mice exhibited significantly decreased expression of Jarid2, coupled with enhanced expression of Znf496, Notch1, Pax7, Fgf1 and Myh3. Expression of myogenin, marker of terminally differentiated myofibers was reduced. Furthermore, reduced muscle fiber cross-sectional area, increased number of fibers with central nuclei and reduced myonuclei number were also observed in CS-exposed animals.
Conclusions: Taken together, the current results provide evidence linking chronic CS exposure and ongoing damage/repair process as well as impaired regenerative capacity in skeletal muscles of CS-exposed study animals.

**References:**

*1. Langen RC, Schols AM, Kelders MC, van der Velden JL, Wouters EF, et al. (2006) Muscle wasting and impaired muscle regeneration in a murine model of chronic pulmonary inflammation. Am J Respir Cell Mol Biol. United States. pp. 689-696.*

*2. Crul T, Spruit MA, Gayan-Ramirez G, Quarck R, Gosselink R, et al. (2007) Markers of inflammation and disuse in vastus lateralis of chronic obstructive pulmonary disease patients. Eur J Clin Invest 37: 897-904.*

# Stress resilience in adolescence and stroke risk in middle age: Swedish register-based cohort study

**Bergh C,[1,2] Udumyan R,[1,3] Fall K,[1,3] Nilsagård Y,[1,4] Appelros P,[5] Montgomery S[1,3,6,7]**

[1]School of Health and Medical Sciences; Örebro University, Örebro, Sweden
[2]Department of Physiotherapy, Örebro University Hospital, Örebro, Sweden
[3]Clinical Epidemiology and Biostatistics, Örebro University Hospital, Örebro, Sweden
[4]Centre for Health Care Sciences, Örebro County Council, Sweden
[5]Department of Neurology, Örebro University Hospital, Örebro, Sweden
[6]Clinical Epidemiology Unit, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden
[7]Department of Epidemiology and Public Health, University College London, UK.

**Objective:** Exposure to psychosocial stress has been identified as a possible stroke risk, but the role of stress resilience, which may be relevant to chronic exposure, is uncertain. We investigated the association of stress resilience with risk of first stroke in middle age. A secondary aim was to examine the extent to which other risk factors might explain associations of stress resilience with stroke.

**Methods:** A cohort of 237 879 male conscripts born between 1952 and 1956 was followed until 2010 using information from Swedish registers. Stress susceptibility was measured at conscription during adolescence using a semi-structured interview with a psychologist. Cox regression estimated the association of stress resilience with first stroke, after adjustment for established stroke risk factors.

**Results:** A total of 3411 diagnoses of first stroke were identified. Lowest stress resilience (21.8%) compared with the highest (23.7%) was associated with increased stroke risk producing unadjusted hazard ratios (HR) with 95% confidence intervals of 1.54 (1.40-1.70). The association attenuated slightly to 1.36 (1.23 to 1.50) after adjustment for markers of socioeconomic characteristics of family background; and after further adjustment for blood pressure, cognitive function and pre-existing cardiovascular disease to 1.25 (1.13 to 1.39). The greatest reduction followed adjustment for BMI and physical working capacity in adolescence, 1.16 (1.04 to1.29).

**Conclusions:** Stress susceptibility may be implicated in the aetiology of stroke. This association is in part explained by poorer physical fitness. Effective prevention might focus on both behaviour and coping with psychosocial

**References**

1. *Egido JA, Castillo O, Roig B et al.* ***(2012).****"Is psycho-social stress a risk factor for stroke? A case-control study"* ***J Neurol Neurosurg Psychiatry*****. 83:1104-1110.**

2. *O'Donnell M, Xavier D, Liu L et al.* ***(2010).*** *"Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study".* ***Lancet. 376: 112-23.***

# Walking the bridge

**M. Ewertsson[1], R. Allvin[2], I.K. Holmström[3], K. Blomberg[1]**

[1]Örebro University, [2]Örebro University Hospital, [3]Mälardalen University, Sweden

**Objective:** A lack of clinical skills among newly qualified nurses can be considered as a patient safety risk. The last decade many Clinical skill laboratories (CSL) have developed and are an arena where nursing students can learn and develop skills.
The aim of this study was to describe nursing students' experiences of learning in CSL in preparation for their clinical practice.

**Design:** The study had a qualitative descriptive design.

**Method:** Data were collected through semi-structured interviews. Sixteen nursing students in the fourth semester of a university in Sweden were included. The interviews were analyzed using qualitative content analysis.

**Results:** The analysis resulted in the overall theme;" Walking the bridge" The CSL constitutes an important bridge between the university and the clinical settings in which students integrate theory and practice as well as develop a reflective stance. The theme was based on the four categories, *"Condition for learning", "Strategies for learning "Tension between learning in CSL versus clinical practice" and "Development of professional and personal competence"*.

**Conclusions:** Through experiences from learning in the CSL students felt prepared for clinical work. However students perceived a tension between learning in the CSL and clinical settings, which they think is negative. This tension can be seen from a positive standpoint as it gives the student further opportunities to reflect on the way to professional competence. In order to promote student learning, it is a pedagogical challenge to increase a joint understanding of students learning needs between CSL and clinical settings.

**Reference:**

1. *Berragan, L., 2011. Simulation: An effective pedagogical approach for nursing? Nurse Education Today 31 (7), 660-663.*

# Comprehensive epigenetic signature in cervical cancer

**Sanja A Farkas[1,2], Nina Milutin-Gašperov[3], Magdalena Grce[3], and Torbjörn K Nilsson[4]**

[1]Department of Laboratory Medicine, Örebro University Hospital, Örebro, Sweden
[2] School of Health and Medical Sciences, Örebro University, Örebro, Sweden
[3]Department of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia
[4]Department of Medical Biosciences/Clinical Chemistry, Umeå University, Umeå, Sweden

**Objective:** The aim of this study was to identify new candidate biomarker genes in cervical squamous cell carcinoma.

**Methods:** We employed the Illumina Infinium HumanMethylation450 BeadChip array to identify genome-wide differential methylation signature in cervical squamous cell carcinoma compared with the cervical intraepithelial neoplasia grade 3 and normal cervical scrapes. The differential methylation was validated by pyrosequencing and external gene expression data sets.

**Results:** The hypo- and hypermethylated genes belonged to different gene ontology clusters; hypomethylated genes in cancer compared with normal cervical tissues were related to the immune system in contrast to the hypermethylated genes that were related to cell development and differentiation. Fifteen of the 24 potential biomarker genes (*ACAN, Clorf114, FBXL7, GYPC, KCNA3, KIF19, LHX8, MIR663, RGS7*, S1PR4, *SORCS1, TBX20, TRIM58, TTYH1*, and *VSTM2B*) have not yet been correlated with any cancer type, while eight (*AJAP1, BARHL2, BOLL, GALR1, PTGDR, ST6GALNAC5, ZIK1*, and *ZSCAN18*) have been implicated in cancers other than cervical cancer. External validation with independent gene expression data sets showed down-regulated expression of 23 potential biomarkers in cancer compared with normal cervical tissues. These results correlated with the hypermethylated state of the genes.

**Conclusion:** The 24 candidate biomarkers that we identified represent several types of mechanisms in the cellular machinery that are epigenetically deregulated, such as membrane receptor function, intracellular signaling, and gene transcription. Understanding of the functional role of DNA methylation alterations in cancer genomes may prove to be clinically applicable in disease diagnostics and prognostics, and may guide the development of new epigenetic therapies.

# Downregulation of human leukocyte antigen class II genes in bone marrow fibroblasts from myelofibrosis

**Fotabe L[1], Kruse R [1], Åström M[2], and Ivarsson M[1]**

[1]Dept. of Clinical Medicine, School of Health and Medical Sciences, Örebro University, Örebro, Sweden.

[2]Dept. of Internal Medicine and Laboratory Medicine, Örebro University Hospital, and School of Health and Medical Sciences, Örebro University, Örebro, Sweden.

**Background:** Primary myelofibrosis is a myeloproliferative neoplasm (MPN) in which there is progressive fibrosis development in the bone marrow, often leading to splenomegaly and worsening anemia. Fibrosis can also develop secondary to other diseases e.g. polycythemia vera and essential thrombocythemia. A fibrotic phenotype is usually well imprinted in stromal fibroblasts in culture. Gene expression of fibroblasts from patients with myelofibrosis has not been investigated before. We have performed a whole genome microarray study on cultured fibroblasts with the aim to gain more knowledge on fibrotic mechanisms.

**Methods:** mRNA isolated from explant cultures of bone marrow fibroblasts from 8 MF patients and 6 control subjects at passages 3-8 were subjected to microarray analysis using the Agilent 4x44K and 8x64K Whole Human Genome Oligo Microarray slides. The data were analysed using GeneSpring version 12.6.

**Results:** A total of 21578 entities were harmonised from both technologies. 176 genes were displayed above the cut off fold change 2.0 and below the false discovery rate (FDR) 0.05 and thereby considered as significantly differentially expressed between the patients and the controls. 57 genes were upregulated and 119 genes were downregulated. The most striking finding was that HLA class II genes were generally downregulated in the patients compared to control subjects.

**Conclusion:** Downregulation of human leukocyte antigen (HLA) class II genes may be part of an immune surveillance escape mechanism in MPNs, as shown for some other cancer forms, and may be relevant for the mechanism of fibrosis development. More studies are needed in order to better understand the pathophysiology of the disease and find new treatment targets.

**Keys words:** Myelofibrosis, primary myelofibrosis, polycythemia vera, essential thrombocythemia, HLA class II genes, fibroblasts.

# How stress may cause thrombosis –novel aspects in epinephrine (adrenalin)-triggered human platelet activation

**Fälker K, Ljungberg L, Grenegård M**

Department of Biomedicine, School of Health and Medical Sciences, Örebro University, SE-701 82 Örebro, Sweden

**Objective**: Platelets activation is negatively regulated by endogenous nitric oxide (NO), prostaglandin $I_2$ ($PGI_2$) and adenosine. The physiological effects of NO and $PGI_2$/adenosine are mediated through elevation of cyclic GMP (cGMP) and cyclic AMP (cAMP), respectively. It is well established that cyclic nucleotides abrogate almost all aspects of platelet activation. We hypothesized that simultaneous activation of different stimulatory signaling pathways may synergize to overcome the effect of cyclic nucleotides.

**Methods**: Isolated human platelets were used for assessing platelet $Ca^{2+}$ mobilization, granules secretion, protein phosphorylation, and aggregation.

**Results**: Thrombin at high doses (0.3 U/ml) easily counteracted platelet inhibition provoked by elevation of either cGMP or cAMP. When cGMP and cAMP were simultaneously elevated, an abrogation of thrombin-induced platelet aggregation was observed. Epinephrine, considered as a "potentiating" activator of platelets, alone had no effect. However, in platelets pre-exposed to cAMP and cGMP-elevating drugs the combination of thrombin (0.3 U/ml) and epinephrine (0.2-20 µM) normalized aggregation. Noteworthy, similar effects were observed in platelet-rich plasma (PRP) and whole blood.
Epinephrine acts through $G_{i/z}$-coupled $\alpha_{2A}$-adrenoceptors and suppresses cyclic AMP synthesis and activates phosphoinositide 3 kinase (PI3-K). Western blot analyses showed that thrombin combined with epinephrine induced a cyclic nucleotide insensitive, PI3-K-dependent, phosphorylation of Akt. Furthermore, the PI3-K inhibitor LY294002 abolished the epinephrine-mediated normalization of aggregation, whereas LY294002 alone had no effect on aggregation caused by thrombin.

**Conclusions**: A positive interplay between thrombin and epinephrine (adrenalin) counterbalances platelet inhibition by concomitant cAMP and cGMP elevation. Epinephrine-evoked PI3K activation plays a key role in this signaling cross-talk.

# The use of the Ussing chamber method to study intestinal permeability after prebiotic stimulation

**Ganda Mall JP[1], Östlund-Lagerström L[1], Löfvendahl L[1], Keita AV[2], Brummer RJ[1], Schoultz I[1]**

[1]Nutrition Gut Brain Interactions Research Centre, School of Health and Medical Sciences, Örebro University, Örebro, Sweden
[2]Clinical and Experimental Medicine, Division of Surgery, Faculty of Health Sciences, Linköping University, Linköping, Sweden

**Objective:** In 1951 the Danish researcher Hans H. Ussing and co-workers developed a technique now known as the Ussing chamber method to study ion transport in frog skin. For more than 50 years has this method been involved in important findings of epithelial physiology regarding secretion, absorption and permeability in almost all kind of tissues, ranging from bladder epithelia to the intestinal epithelium. Our aim is to use this method to study the intestinal barrier's capability to withstand permeability-inducing stress after prebiotic treatment.

**Method**: Biopsies from the intestine are mounted in the Ussing chamber to study permeability but it is also important to know how viable they are. The Ussing chamber system gives three electrophysiological readings which are important measurements of the tissues viability. Transepithelial electrical resistance (TER) is a measurement of the intestinal barrier integrity. This parameter is calculated from the short-circuit current (Isc) and the potential difference (PD). Isc is a measurement of ion flux while PD is a measurement of the active transport by the epithelia and thus the most important parameter for viability. For permeability studies are two markers added to the mucosal side of the chamber and measured in samples taken from the serosal side of the chamber at different time points.

**Results:** We have established two Ussing chamber systems in our lab and are right now optimizing the method in order to get as viable biopsies as possible and to find the optimal concentration of our permeability-inducing stressor corticotrophin-releasing hormone.

# The effect of 17β-estradiol on NLRP3 inflammasome and inflammatory cytokines in HUVEC and AOSMC

**George Gkoumas[1, 2], Geena Paramel Varghese[2], Karin fransén[2], Allan sirsjö[2]**

[1]Department of Medical Laboratories, Technological Educational Institute of Athens (TEI), Greece.
[2]School of Health and Medical Sciences, Örebro University, Sweden.

**Objectives:** Gender difference in inflammatory disease such as CVD has been reported both in human and experimental animals. The gender specific role of estrogen has been reflected in vascular injury response. Since observational studies have shown substantial benefits of estrogen in reducing the relative risk of Coronary heart disease (CHD) by 50%, we examined the effects of estradiol on NLRP3 inflammasome components and inflammatory cytokines in vascular cells.

**Methods:** AOSMC and HUVEC were grown in estrogen free medium before the assay. Due to the low basal expression of NLRP3 inflammasome components in HUVEC, the effect of estrogen was examined by stimulating the cells with TNFα. Real time PCR and ELISA was performed to analyze the mRNA expression and IL-1β, IL-6 and IL-8 protein.

**Results:** Exogenous 17β -estradiol significantly down regulated the expression of *NLRP3, caspase-1* and *IL-1β* in HUVEC and AOSMC. In AOSMC, the treatment of estrogen resulted in a moderate reduction of IL1β release and significant reduction of IL-6 and IL-8 levels.

**Conclusions:** These studies demonstrate that estrogen treatment reduces vascular inflammation by down regulating NLRP3 inflammasome and reducing proinflammatory cytokine in HUVEC and AOSMC.

**References:**

1. *Varghese G, Fransén K, Hurtig‐Wennlöf A, Bengtsson T, Jansson J, Sirsjö A.* ***(2013)****."Q705K variant in NLRP3 gene confers protection against myocardial infarction in female individuals."* ***Biomedical Reports 1(6): 879-882.***

2. *Xing D, Susan N, Yiu-Fai C, Fadi H.* ***(2009).*** *"Estrogen and mechanisms of vascular protection."* ***Arterioscler Thromb Vasc Biol 29(3): 289-295.***

# IL-1/TLR Signaling Inhibitors in Microscopic and Ulcerative Colitis: Immunopathogenic Markers of Active Disease and Remission

**Sezin Günaltay*, Nils Nyhlin †, Ashok Kumar Kumawat*, Curt Tysk †, Johan Bohr †, Olof Hultgren ‡, and Elisabeth Hultgren Hörnquist***

*Department of Clinical Medicine, School of Health and Medical Sciences, SE-701 82 Örebro University, Örebro, Sweden
† Department of Medicine, Division of Gastroenterology, Örebro University Hospital, School of Health and Medical Sciences, Örebro University, SE-701 85 Örebro, Sweden
‡ Department of Microbiology and Immunology, Örebro University Hospital, SE-701 85 Örebro, Sweden

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the intestine consisting of ulcerative colitis (UC) and Crohn's disease, as well as microscopic colitis (MC, collagenous colitis (CC) and lymphocytic colitis (LC)). The etiology is unknown but thought to involve an abnormal immune response to luminal agents. Innate immune recognition of microbial products via Toll-like receptors results in increased expression of inflammatory genes, which must be controlled to ensure sufficient clearance of pathogens but simultaneously avoid extensive tissue damage. We compared expressions of IRAK-2, IRAK-M, IL-37 and microRNAs (miR)-146a, -155 and -21 in colon biopsies of CC, LC and UC patients in active disease or remission with non-inflamed controls by qRT-PCR. IRAK-M expression was increased in LC patients with active disease in histopathological remission (LC-HR; p=0.02) and UC (p=0.01) patients, but no differences in IRAK-2 expression were detected compared to controls. miR-146a, -155 and -21 expressions were increased in LC-HR (p=0.04, 0.07, and 0.004) and UC (p=0.02, 0.04 and 0.03) patients. Active UC patients had significantly enhanced miR-146a and miR-21 expressions compared to UC-R (p=0.01 and 0.04). Likewise, active CC patients showed significantly increased expression of miR-155 (p=0.003) and miR-21 (p=0.006). IL-37 expression was decreased in both CC (p=0.03) and LC (p=0.04) patients with a similar trend in UC patients, albeit not statistically significant, whilst it was increased in UC remission (UC-R) patients compared to controls (p=0.02) and active UC (p=0.001). The identification of differentially expressed miRNA, IL-37 and IRAK-M suggest different pathophysiologic mechanisms in various disease stages.

# INCREASED EXPRESSION OF T CELL RECRUITING CHEMOKINES IN THE COLONIC MUCOSA OF MICROSCOPIC COLITIS PATIENTS

**Sezin Günaltay*, Nils Nyhlin †, Ashok Kumar Kumawat*, Curt Tysk †, Johan Bohr †, Olof Hultgren ‡, and Elisabeth Hultgren Hörnquist***

*Department of Clinical Medicine, School of Health and Medical Sciences, SE-701 82 Örebro University, Örebro, Sweden
† Department of Medicine, Division of Gastroenterology, Örebro University Hospital, School of Health and Medical Sciences, Örebro University, SE-701 85 Örebro, Sweden
‡ Department of Microbiology and Immunology, Örebro University Hospital, SE-701 85 Örebro, Sweden

Microscopic colitis (MC), comprising collagenous colitis (CC) and lymphocytic colitis (LC), is a common cause of chronic diarrhea. In this study we compared gene expression of chemokines and their receptors in colon biopsies from MC patients in active disease or in histological remission (CC/LC-HR) with controls by qRT-PCR. The Th1-associated chemokines CCL2/MCP-1, MIP-1β/CCL4, MIG/CXCL9, IP10/CXCL10, I-TAC/CXCL11, the Th2-associated chemokine MDC/CCL22, the Th17-associated chemokine IL-8/CXCL8 and cytotoxic T cell-associated fractalkine/CX3CL1 expressions were increased in both active CC and LC patients compared to controls. The Th1/Th2-associated chemokine RANTES/CCL5 expression increased only in LC patients whereas the Th1-associated chemokine MIP-1α/CCL3 expression increased in CC patients only compared to controls. Both CC and LC patients had increased receptor expressions (ligand(s) in parenthesis): CCR3 (CCL5, 7), CCR4 (CCL5, 22), CXCR1 and 2 (CXCL8), whilst, CX3CR1 (CX3CL1) and CCR2 (CCL2, 7) expressions were increased only in CC patients. CC-HR and LC-HR patients showed increased expression of CCR3, CXCR2 and CX3CR1, whereas CCL22, CXCL9, CXCL11, CX3CL1, CCR4, and CXCR1 expressions were increased in LC-HR patients compared to controls. Contrary, CCL2, 3, 22, CXCL8, CCR2, 4, 5, CX3CL1, CXCL9, 10, 11 and CXCR3 expressions decreased in CC-HR patients compared to CC patients. This study shows similar mucosal expression of T cell recruiting chemokines in CC and LC but altered expressions in different disease stages. These findings are important for elucidation of MC immunopathogenesis and identification of potential therapeutic candidates.

# Reduced TLR5, NOD2, NLRP3 mRNA expression and inflammasome-dependent caspase-1 activity in cystic fibrosis bronchial epithelial cells with the ΔF508 mutation

**Shahida Hussain[1], Godfried M. Roomans[2] and Eva Särndahl[2]**

[1]School of Health and Medical Sciences, Örebro University, Örebro, Sweden
[2]Department of Medical Education, Örebro University, Örebro, Sweden

Cystic fibrosis (CF) is associated with chronic airway infections caused by a number of bacteria, notably *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The innate immune system of the airways has evolved as a system for identifying and controlling microbial infections and is important in the pathophysiology of CF. The aim of the current study was to investigate the mRNA expression of Toll-like receptor (TLR)5 and NOD-like receptors (NLR), as well as the inflammasome activity in CF bronchial epithelial cells. This was done by detecting TLR5, NOD1, NOD2 and NLRP3 mRNA expression using QRT-PCR and caspase-1 activity by flow cytometry in CF (CFBE) and normal (16HBE) bronchial epithelial cells under unstimulated conditions, as well as after flagellin treatment. Unstimulated CFBE cells displayed lower mRNA expression of NOD2 and NLRP3, but no difference in the expression of NOD1 was detected compared to wild-type 16HBE cells. Whereas flagellin, which is a known dual ligand for TLR5 and NLRC4, caused an increased mRNA expression of NOD1, NOD2, and NLRP3 in 16HBE cells, a time-dependent decrease in the expression of these receptors was detected in CFBE cells. TLR5 but not NLRC4 mRNA was detected in the CFBE cells, which indicates that flagellin most probably signals through TLR5 to stimulate the innate immune system in the airway epithelium. Furthermore, under basal conditions, CFBE cells showed lower caspase-1 activity than normal 16HBE cells. Flagellin *per se* had no effect on the caspase-1 activity. The overall lower caspase-1 activity in CFBE cells indicates downregulated inflammasome-dependent caspase-1 activation than 16HBE cells. Apparently, the immune response to flagellin in CF cells is weaker than that in normal cells, which allows other bacteria to colonize CF airways.

# C10X Polymorphism in the *CARD8* Gene is Associated with Bacteremia

**Berhane Asfaw Idosa** [1*], **Berolla Sahdo**[1], **Ermias Balcha** [1], **Anne Kelly** [1], **Bo Söderquist** [2, 3] **Eva Särndahl** [1, 3*]

[1]Department of Clinical Medicine, School of Health & Medical Sciences, Örebro University, SE-701 82 Örebro, Sweden
[2]Department of Laboratory Medicine, Medical Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden
[3]Faculty of Medicine and Health, Örebro University, SE-701 82 Örebro, Sweden

**Objective:** The NLRP3 inflammasome is an intracellular multi-protein complex that triggers caspase-1 mediated maturation of interleukin-1β (IL-1β); one of the most potent mediators of inflammation and a major cytokine produced during severe infections, like sepsis. However, the excessive cytokine levels seem to stage for tissue injury and organ failure, and high levels of IL-1β correlates with severity and mortality of sepsis. Instead, recent data suggest caspase-1 to function as a guardian against severe infections. CARD8 has been implied to regulate the synthesis of IL-1β via interaction to caspase-1. In recent years, polymorphism of *CARD8* (C10X) *per se* or in combination with *NLRP3* (Q705K) has been implicated with increased risk of inflammation. The aim was to investigate the correlation of these polymorphisms with severe blood stream infection.

**Methods:** Human DNA was extracted from blood culture bottles that were found to be positive for microbial growth (*i.e.* patients with bacteraemia). Polymorphisms Q705K in the *NLRP3* gene and C10X in the *CARD8* gene were genotyped using TaqMan genotyping assay. The results were compared to healthy controls and to samples from patients with negative cultures.

**Results:** The polymorphism C10X was significantly over-represented among patients with bacteraemia as compared to healthy controls, whereas patients with negative blood culture were not associated with a higher prevalence. No association was observed with polymorphism Q705K of *NLRP3* in either group of patients.

**Conclusion:** Patients carrying polymorphism C10X in the *CARD8* gene are at increased risk of developing bacteraemia and severe inflammation.

**References**

1. *Sahdo B, Fransèn K, Asfaw Idosa B, Eriksson P, Söderquist B, Kelly A, and Särndahl E. Cytokine profile in a cohort of healthy blood donors carrying polymorphisms in genes encoding the NLRP3 inflammasome. PLoS ONE. 2013 Oct: 8(10):e75457.*

2. *Verma D, Lerm M, Blomgran Julinder R, Eriksson P, Söderkvist P, Särndahl E. Gene polymorphisms in the NALP3 inflammasome are associated with interleukin-1 production and severe inflammation: Relation to common inflammatory diseases? Arthritis Rheum. 2008 Mar;58(3):888-894.*

# Lysine gingipain could be involved in regulating CXCL8 in *Porphyromonas gingivalis* infected monocytes at both, gene and protein levels

**Jayaprakash Kartheyaene, Khalaf Hazem, Sirsjö Allan, Bengtsson Torbjörn**

Department of Biomedicine, School of Health Sciences, Örebro University, Örebro

**Objective:** Gingipains are cysteine-like trypsin proteases produced by *Porhyromonas gingivalis*, an oral pathogen, that can cleave various proteins including cytokines thereby reducing the efficacy of innate immune response which presents the disease as a chronic low grade infection. The gingipains are of two types – Arginine (Rgp) and Lysine (Kgp). Here, in this study we analyze the ability of various strains on *P. gingivalis* on their ability to regulate CXCL8 expression at gene levels and also study their potency at cleaving CXCL8 at protein levels from the culture supernatants. Making use of mutants could provide an insight into the the specificity of the proteolytic gingipain activity.

**Methods:** THP-1 cells were stimulated with wild type strains ATCC 33277, W50 and isogenic mutants of W50 –Arginine- gingipain mutant E8 and lysine- gingipain mutant K1A for 24 hours. Supernatants were measured for CXCL8 by enzyme – linked immunosorbant assay. mRNA expression of CXCL8 was quantified using real-time polymerase chain reaction. Bacterial- monocyte interaction and invasion was demonstrated by confocal microscopy using FITC- labeled bacteria.

**Results:** The W50 strain and its isogenic arginine-- gingipain mutant E8 treated cells showed very minimal levels of CXCL8 in the cell culture supernanatants whereas, K1A treated cells showed the highest level of CXCL8 followed by the ATCC 33277 strain. The mRNA expression followed the same pattern of CXCL8 expression. Confocal images show that the ATCC strain has invaded the monocytes which were later retrieved on blood agar by the antibiotic protection assay test after 4 hours of bacterial – monocyte interaction.

**Conclusions**: The Kgp down regulates CXCL8 at both gene and protein levels which appears to be antagonistic to the effects of Rgp. The ATCC strain is not as potent as W50, a clinical isolate. Presence of viable bacteria within monocytes could also imply that the bacteria could be transferred to different sites of the body resulting in systemic dissemination from the oral sites.

**Reference:**

1. *Guo Y, Nguyen KA, Potempa J.* ***(2010).*** *Dichotomy of gingipains action as virulence factors: From cleaving substrates with the precision of a surgeon's knife to a meat chopper-like brutal degradation of proteins*. **Periodontol 2000; 54:15-44.**

## Wound healing

**Koskela Anita och Ivarsson Mikael.**

School of Health and Medical Sciences, Örebro University, Örebro, Sweden
Clinical research Center, Örebro University Hospital, Örebro, Sweden

**Objective:** The aim was investigate how keratinocytes regulate fibroblast genes involved in extracellular matrix remodelling, in a more *in vivo*-like condition. We used a keratinocyte-fibroblast organotypic skin culture model to elucidate possible anti-fibrotic effect of keratinocytes during epidermal generation.

**Methods:** The organotypic skin cultures were grown for up to 7 days. To study how epidermal regeneration progressed, the organotypic cultures were snap-frozen and sectioned for morphology as well as staining for epidermal differential markers keratin 10, keratin 14, involucrin and loricrin. Expression of 12 genes important for the modulation of the extracellular matrix were analysed with real-time PCR.

**Result:** The organotypic skin culture formed a skin equivalent within 7 days. The stratified keratinocyte layer expressed the late epidermal differentiation markers keratin 10, involucrin, and loricrin. The basal layer expressed the keratin 14 as expected.
A set of twelve experiments were performed analysing fibroblast gene expression. 11 out of 13 genes were significantly regulated by keratinocytes, either in the presence or absence of TGF.

**Conclusion:** Our results demonstrate mechanisms by which keratinocytes affect fibroblasts to act catabolically on the extracellular matrix in the reepithelialisation process. This adds understanding to the observations that reepithelialisation and epithelial transplantation reduces scar formation.

**Referenses:**

1. *Regulation of fibroblast gene expression by keratinocytes in organotypic skin culture provides possible mechanisms for the antifibrotic effect of reepithelialization. Koskela A, Engström K, Hakelius M, Nowinski D, Ivarsson M. Wound Repair Regen. 2010 Sep-Oct; 18 (5):452-9.*

# Occupational exposure to HIV among healthcare workers in Uganda

**Kumakech E[1,2]. Achora S[2]; Berggren V[3,4], Bajunire F[5].**

[1]Dept. of Healthcare Sciences, Orebro University, Orebro, Sweden.
[2]Dept. of Nursing, Mbarara University of Science and Technology, Mbarara, Uganda
[3]Divi. of Global Health/IHCAR, Karolinska Institute, Stockholm, Sweden
[4]Dept. of Health Sciences, Kristianstad University, Kristianstad, Sweden
[5]Dept. of Community Health, Mbarara University of Science and Technology, Mbarara, Uganda

**Objective:** Occupational exposure to HIV presents a low but potential risk of HIV infection to healthcare workers especially in high HIV prevalent areas. Therefore the objective was to determine the prevalence of occupational exposure to HIV, the circumstances and predisposing factors, and post exposure management.

**Methods:** Questionnaires were distributed to 224 participants comprising of 98 healthcare workers and 126 clinical students in Mbarara hospital Uganda. Collected data were analyzed with descriptive and chi square statistics using SPSS 15.0.

**Results:** Of the 224 participants surveyed, 19,2% of the participants reported ever been exposed to HIV from their clinical work. Routes of exposure included muco-cutaneous contamination (10.3%) and stick injuries (8,9%). Clinical procedures for exposure included delivering a baby (19,5%), cannulation (16,3%), phlebotomy (11,3%), giving injection (8,1%) and conducting surgery (7,7%). The most affected groups were nurses–midwives and clinical students especially for exposure through injuries.

Lack of protective devices and recapping of needles were the commonest predisposing factors. Exposures were underreported and hence low access to post exposure prophylaxis (PEP).

**Conclusion:** Occupational exposure to HIV remains a frequent occurrence particularly among student nurses-midwives, despite being avoidable. Its prophylactic treatment is hampered by poor reporting and investigation of exposures, and poor access to PEP. Strict adherence to universal precaution and proper handling of occupational exposure to HIV should be encouraged.

**References:**

1. *Sagoe-Moses, C., Pearson, R.D., Perry, J. & Jagger, J.* ***(2001).*** *Risks to healthcare workers in developing countries.* ***New England Journal of Medicine.*** *345 (7): 538–41.*

# Healthcare providers and policy makers' perceptions of an integrated approach to delivery of HIV and cervical cancer screening services in Uganda

**Kumakech E[1,2]. Anderson S[3], Wabinga H[4], Berggren V[5,6]**

[1]School of Health and Medical Sciences, Orebro University, Orebro, Sweden.
[2]Dept. of Nursing, Makerere University College of Health Sciences, Kampala, Uganda
[3]Dept. of Laboratory Medicine, Orebro University Hospital, Orebro, Sweden
4Dept. of Pathology, Makerere University College of Health Sciences, Kampala, Uganda
[5]Dept. of Health Sciences, Lund University, Lund, Sweden
[6]Div. of Global Health/IHCAR, Karolinska Institute, Stockholm, Sweden

**Objective:** HIV positive women have an increased risk of developing cervical cancer (CC) compared to the HIV negative women. Therefore, the study objective was to understand the healthcare providers (HCP) and policy makers (PM) perceptions of an integrated approach to delivery of HIV and CC screening services in Uganda.

**Methods:** This was a qualitative study. Data were collected from 16 participants comprising of HCP and PM using individual interviews. The participants were purposively selected, had diverse experience and were from the different levels of healthcare in Uganda.

**Results:** All the participants perceived the integrated approach to provide manifold benefits. Compassion for the benefices was seen, especially related to HIV positive women. Benefits to women mentioned included access to more health services, proper schedule for CC re-screening, early diagnosis and treatment, referral and saving on transportation cost and time. Other benefits were mentioned for the men, HCP and the government.
But there also emerged worries about integration. Expressed was the worry that integration would increase workload for HCP, HIV stigma will affect CC screening, and women's waiting time at the health facility will be prolonged.

**Conclusion:** Although an integrated approach to delivery of HIV and CC screening service was perceived to offer manifold benefits but the existing weaknesses in the health systems needs to be addressed before considering integration.

**References:**

1. *Hawes SE, et al.* ***(2003).*** *Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with HIV 1 and 2 infections.* ***J Infect Dis****. 188:555–63.*

# The importance of a mentoring program in strengthening female youths and promoting female mental health

**Madelene Larsson[1], Camilla Pettersson[1], Therése Skoog[2], Charli Eriksson[1]**

[1]School of Health and Medical Sciences, Örebro University, Örebro, Sweden
[2]School of Law, Psychology and Social work, Örebro University, Örebro, Sweden

Young females report overall lower moods, more negative self-concepts and more psychosomatic disorders compared to boys. Tjejzonen (the Girl zone) is a NGO in Sweden targeting female youths to prevent mental health problems and to strengthening girls´ self-esteem, confidence and trust. Dyads of a young girl 12-25 years old, the Little sister (LS), and a ten year older girl, the Big sister (BS) (mentor), meet twice a month to talk about issues important to the LS. A BS's role is to be someone girls can talk to, be inspired from and be supported by.

**Objective:** The study is examining the importance of the mentoring program. What do this mentoring program and the relationship mean to the LSs?

**Methods:** As part of a larger study, five LSs who had met their BSs for at least 6 months participated in semi-structured interviews analyzed using inductive qualitative content analysis.

**Results**: The LS were strengthened by the relationships. The BS had limited information about the LS before they met and the LS were in control of what information to share with their mentors. The LS had strong positions in the mentoring program. They were taken seriously and treated with respect. The BS did not judge them and were good listeners. The LS also expressed that the relationships contributed to their personal development, increased the self-insights and constructive handling of feelings.

**Conclusions**: The mentoring program can contribute to increased empowerment among female youth. This will be further analyzed in the on-going longitudinal study.

# Integration and methylation status of E2-binding sites in HPV 16 in vulvar and vaginal carcinoma.

**Lillsunde Larsson G [1,2], Helenius G [1,2], Andersson S [1,2], Sorbe B [3], Karlsson M [1,2]**

[1]Department of Laboratory Medicine, Örebro University Hospital, Örebro Sweden;
[2] School of Health and Medical Sciences, Örebro University, Örebro Sweden;
[3] Department of Oncology, Örebro University Hospital, Örebro Sweden.

**Objectives:** Integration of the viral DNA into the human genome is proposed being an event preceding the transmission from premalignant lesions to carcinoma. Upon integration, the regulatory protein E2 is lost and high levels of the viral onkoproteins E6 and E7 are expressed. However, studies have shown that occasionally the viral genome is kept in episomal state and no, or only partial, integration occurs.
We have studied an alternative method for tumor development, where we hypothesize that methylation of E2-binding sites could deregulate E6 and E7 expression, by stopping E2 executing its regulatory role.

**Methods:** Integration status was measured with realtime PCR for HPV16 E2 and E6, and calculated by dividing copy numbers of E2 with E6. Levels of methylation for E2BS3/4 were performed using pyrosequencing. Tumor DNA was bisulfide treated followed by PCR. Pyrosequencing of 5 positions in E2BS3/4 were analyzed and results divided into low, medium and high scores.
Clinical data were retrieved from the dep. of oncology at Örebro university hospital.

**Results:** Most vaginal and vulvar tumors (n=57) had the viral DNA in episomal state (63 %) and showed low methylation levels (75%). High degree of methylation was only seen in tumors having episomal viral DNA. Integrated tumors were not highly methylated. High viral load in the vulvar cohort (n=31) was associated with worse survival. ($p=0.047$). The vaginal recurrence rate was significantly associated with high methylation ($p=0.015$).

**Conclusion:** Our results indicate that methylation of E2-binding sites could be an alternate mechanism for tumor development.

# Evaluation of the novel Anyplex$^{tm}$ II HPV28 genotyping assay for diagnosis and typing of HPV from archival clinical samples

**Helenius G[1,2], Lillsunde-Larsson G[1,2], Andersson S[1,2], Unemo M[1,2], Karlsson MG[1,2]**

[1]Department of Laboratory Medicine, Örebro University Hospital, Örebro Sweden;
[2] School of Health and Medical Sciences, Örebro University, Örebro Sweden;

**Objectives:** In this study we have evaluated a new multiplex assay, Anyplex™ II HPV28, based on a modified real-time PCR and melting curve analysis. Anyplex™ II HPV28 was compared to a reference method which is a type-specific real-time PCR.

**Methods:** 99 FFPE samples from patients with a clinical suspicion of HPV infection at the Department of Pathology, Örebro University Hospital were included.
Anyplex™ II HPV28 (Seegene) detects HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 69, 70, 73, 82 and beta-globulin in 2 multiplex reactions. All tests were run on a CFX96TM Real-time PCR System (BioRad). The reference method detects HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and betaglobulin and were analysed on the 7900 HT Real-Time PCR System (Life Technologies).

**Results:** When comparing the genotypes detectable by both assays, 63% of the samples were HPV positive with Anyplex™ II HPV28 compared to 67% with the reference method. Including all genotypes, Anyplex™ II HPV28 detected HPV in 72% of the samples. Both methods detected HPV16 in 30/99 and HPV18 in 8/99. Notable was that Anyplex™ II HPV28 detected HPV 56 in 11/99 samples compared to 1/99 with the reference method.

**Conclusions:** Anyplex™ II HPV28 appeared effective for genotyping of HPV in archival clinical samples and could be used in a clinical setting.

# Factors influencing adherence to hygiene routines in community care - the viewpoint of Medically Responsible nurses (MRN's) in Sweden.

**M Lindh[1,2], A Kihlgren[1], K-I Perseius[2,3]**

1. School of Health and Medical Science, Örebro University. Örebro, Sweden.
2. Department of Health Care Sciences, Ersta Sköndal University College
3.Department of Neurobiology, Care Sciences and Society, Karolinska Institutet. Stockholm, Sweden.

**Objective**: The aim of the study was to describe factors influencing adherence to hygiene routines in Swedish municipality care from the perspective of the MRN's. Research in infection control in the municipality is rare and therefore of interest.

**Methods**: A web-based questionnaire was sent to all available (n=268) MRNs in Sweden with a reply rate of 124 MRNs (46%). Two open questions were analyzed with content analysis.

**Result:** Four categories were found:
*Resources* were referring to economic priorities as possibilities in consultations of nurse specialist competence and time given for enhancing knowledge. Resources also meant; access and availability to material and equipment.
*Management* interest in putting hygiene on the agenda was important. It was made by clear and consistent communication; highlighting guidelines, organizing PPI's, and giving feedback to staff.
*Staff:* Nurses have a key role in upholding sufficient hygiene routines in community care. Influence on adherence to hygiene routines were influenced by educational level but equally influenced by staff interest in the issue.
*External factors*: media focus on epidemic outbreaks was helpful when claiming for resources. Government interest in infection control was also influenced by media. The community reform from 1992 resulting in restrictions in institutional environment in elderly care towards home like environment was mentioned as one obstacle to infection control.

**Conclusion:** MRNs and RNs have an important role in keeping a high hygiene standard in municipality care.

**Reference:**

1. *Lindh M., Kihlgren A., Perseius KI. Factors influencing compliance to hygiene routines in community care – the viewpoint of Medically Responsible Nurses in Sweden. Scandinavian Journal of Caring Sciences. 2012. Doi: 10.1111/j.1471-6712.2012.01022.*

# Computational system analysis of tyrosine metabolic pathway in fibroblast cells as a tool for studying the biochemical background of mental diseases

**Logotheti M.[1,2,3], Pilalis E.[2], Shemirani I.M.[1], Chatziioannou A.[2], Venizelos N.[1], Kolisis F.[3]**

1. Neuropsychiatric Research Laboratory, Dept. of Clinical Medicine, Örebro University, Örebro, Sweden.
2. Metabolic Engineering and Bioinformatics Group, Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou ave., 11635, Athens, Greece.
3. Laboratory of Biotechnology, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece.

It is established that amino acids involved in neurotransmitter biosynthesis, as for instance tyrosine, a precursor of the neurotransmitter dopamine, play a central role in mental health diseases as Schizophrenia, Bipolar Disorder, Autism and ADHD. In this direction, metabolic network simulations are emerging as valuable tools for the description of complex biochemical systems, such as the metabolic processes that take place in brain cells. In this work we describe an approach to construct such a model, which is based on the Biochemical Systems Theory (BST).
This mathematical and computational framework employs power-law functions for the representation of the metabolic pathways. It has been shown that biological systems, characterized by high variability and nonlinearity, are often well modeled by power laws. The greatest advantage of BST models over traditional kinetic models is that in BST models the effect of any given system component on any given process is uniquely described by one kinetic order plus one rate constant for the overall turn-over rate of the process. Therefore, model design is greatly simplified. In this work we present the construction of a model of tyrosine metabolism in fibroblasts, consisting of a number of important selected reactions.
The choice of this specific cell type has been done for two main reasons: on one hand, tyrosine transport mechanisms in fibroblasts and in the blood-brain barrier are similar and on the other hand, transport kinetics from these cells are available in healthy and in disease conditions.

**References:**

1. *Persson M.L., Johansson J., Vumma R., Raita J., Bjerkenstedt L., Wiesel F.A., Venizelos N., Aberrant amino acid transport in fibroblasts from patients with bipolar disorder. Neurosci. Lett, 2009.* ***457****(1): p. 49-52.*

2. *Qi Z., Miller G.W., and Voit E.O., Computational systems analysis of dopamine metabolism. PLoS One, 2008.* ***3****(6): p. e2444.*

# Experiences of using mixed methods for testing validity and reliability in a clinical tool on Patient Participation

**Luhr K [1, 2]. Marie Holmefur[1], Ulrica Nilsson[1], Ann Catrine Eldh[3, 4]**

[1] School of Health and Medical Science, Örebro University
[2]Family Medicine Research Centre, Örebro County Council
[3] Högskolan Dalarna
[4] NVS, Karolinska Institutet

**Objective:** Patient participation is a health care priority. A clinical tool has recently been developed containing three sections, for the patient to 1) define, 2) prioritize, and
3) evaluate patient participation using 12 recurrent items.
One item; 'being listened to as a patient by the health care staff' illustrates the test.

**Methods:** Mixed methods were used to evaluate validity and reliability. Content and face validity was evaluated by Think Aloud interviews with researchers (n=10) and patients (n=11) experienced in patient participation, later analysed with content analysis. Patients (n=110) with chronic obstructive pulmonary disease and/or heart failure responded to the different sections of the tool before and after multiple health care contacts. Reliability coefficients were calculated using Kappa (κ), weighted Kappa ($\kappa_w$) and Prevalence and Bias Adjusted Kappa (PABAK).

**Results:** The item was considered relevant, even essential, for a patient participation tool and to capture reciprocity, important in patient participation. The item was easy to understand and of interest to respond to.

Test-retest reliability for the item in section 1 showed κ=0.13, PABAK of 0.67. In section 2 and 3 $\kappa_{w=}$0.33 and 0.56 and PABAK=0.68 and 0.73 respectively. Satisfactory agreement was demonstrated.

**Conclusions:** By mixed methods, we found that the item 1) captured the concept, and 2) was of clinical relevance for patients in health care interactions, and 3) showed stability. This was also the case for the other items of the tool.

The findings support the benefits of combining qualitative and quantitative methods when testing a clinical tool.

# Evaluation of the multiplex AmpliSens HCV/HBV/HIV-FRT real-time PCR for simultaneous qualitative detection of Hepatitis C RNA, Hepatitis B DNA and HIV RNA in clinical plasma samples

**Malm K[1], Unemo M[1], Thulin Hedberg S[1], Kireev D[2], and Andersson S[1]**

[1]Department of Laboratory Medicine, Örebro University Hospital, Örebro, Sweden
[2]Central Research Institute for Epidemiology, Moscow, Russia

**Objectives:** Human donors of tissues and organs are obliged to undergo analysis for blood transmitted infections. Serological assays are used, but for ideal sensitivity these assays are supplemented with a nucleic acid amplification test (NAAT). We have evaluated the multiplex AmpliSens HCV/HBV/HIV-FRT real-time PCR for simultaneous qualitative detection of HCV RNA, HBV DNA and HIV RNA in clinical plasma samples.

**Methods**: Clinical plasma samples with known concentrations of HCV (range: 25 - $4.9\times10^6$ IU/mL), HBV (20 – $7.6\times10^4$ IU/mL) and HIV (34 – $4.7\times10^5$ c/mL); samples from virus-negative blood donors (n=100) and WHO reference standard samples from NIBSC (National Institute for Biological Standards and Control, South Mimms, United kingdom) were tested, in 10-fold dilutions. Nucleic acid was isolated from 1 mL plasma on the MagNA Pure Compact with Total Nucleic Acid Isolation kit I-Large Volume (Roche Diagnostics) or MagNA Pure LC with Total Nucleic Acid Isolation kit -Large Volume (Roche Diagnostics) The multiplex AmpliSens HCV/HBV/HIV-FRT real-time PCR (Central Research Institute of Epidemiology, Moscow, Russia) was run on a Rotor-Gene Q PCR instrument (Qiagen).

**Results**: 93 samples with various viral loads of HCV (n=34), HBV (n=30) and HIV (n=32), have been analyzed. Only three samples with very low concentrations of HCV (<25-59 IU/mL) were false negative, and no false positive samples have been found.

**Conclusion**: The multiplex AmpliSens HCV/HBV/HIV-FRT real-time PCR proved to be highly sensitive and specific. Accordingly, this rapid, technically simple and low cost assay might be effectively used for screening of human donors as well as for other diagnostic purposes.

# Lithium-induced hyperparathyroidism (LHPT): prevalence and epidemiology

**Adrian Meehan[1], Mats Humble[2], Payam Yazarloo[3], Johannes Järhult[4], Göran Wallin[5].**

[1]Dept. of Geriatric Medicine, Örebro University Hospital, Örebro, Sweden.
[3]Dept. of Psychiatry, Örebro University Hospital, Örebro, Sweden.
[3]Dept. of Psychiatry, Ryhov County Hospital, Jönköping, Sweden.
[4]Dept. of Surgery, Ryhov County Hospital, Jönköping, Sweden.
[5]Dept. of Surgery, Örebro University Hospital, Örebro, Sweden.

**Objective**: While the prevalence of hyperparathyroidism (HPT) is 1‰ in the general population, 2-3% in postmenopausal women, HPT is presumed to be considerably higher in patients treated with Lithium, principally with bipolar disease. Earlier studies, based on small populations, estimate the prevalence of LHPT around 10-30%[1]. Our study aims to calculate accurately LHPT prevalence on a large Swedish population.

**Method**: Health records of patients on lithium treatment were examined from the respective outpatient psychiatric clinics in Örebro, Lindesberg and Hallsberg in Örebro County and for the catchment area of Ryhov County Hospital, Jönköping. The total catchment area is approximately 400,000. Biochemical values and necessary surgical details were noted.

**Result**: A power analysis according to WW Daniel was calculated; 412 patients currently participate (245 ♀/167 ♂), treated on average 13.5yrs (range 1-46yrs). A multifaceted definition of LHPT was formulated[2]. Accordingly, 82 patients (19.9%) were identified with LHPT. Additionally, a fifth (20.8%) showed tendencies towards HPT in lab tests. Only five patients (≈1%) had undergone parathyroidectomy. A third (33.6%) had pathological thyroid test results. Six cases (≈1.5%) of reported lithium-induced nephropathy. Suicidality was ascertained in 14% of cases. Seven patients died during study period.

**Conclusion**: In this unique study, soon to be submitted, the proposed prevalence of LHPT is as high as 20%. The research group has published standard recommendations on management of these patients[2]. Continual, specific follow-up is required. As lithium treatment is often life-long, surgery should be considered for those meeting LHPT criteria in order to improve psychiatric well-being and multi-organic protection.

**References**

1. *Järhult J, Ander S, Asking B, Jansson S, Meehan A, Kristofferson A, Nordenström J. Long-term results of surgery for lithium-associated hyperparathyroidism. British Journal of Surgery 2010;97:1680-1685.*

2. *Humble M, Meehan A. Rådgivande anvisningar för handläggning av kalciumbalansen hos litium-behandlade patienter. Tidskriften för Svensk psykiatri #3, september 2013.*

# Pre-injury beta-blockade is protective in isolated traumatic Brain injury

**Shahin Mohseni, Peep Talving, Göran Wallin, Olle Ljungqvist, Louis Riddez**

Department of Surgery, Örebro University Hospital
Department of Trauma and Acute Care Surgery, Karolinska University Hospital

**Introduction:** The purpose of this study was to investigate the effect of pre-injury beta-blockade in patients suffering isolated severe traumatic brain injury (TBI). We hypothesized that beta-blockade prior to TBI is associated with improved survival.

**Methods:** The trauma registry of an urban academic trauma center was queried to identify patients with an isolated severe TBI between 1/2007 and 12/2011. Isolated severe TBI was defined as an intracranial injury with an abbreviated injury scale of (AIS) ≥3 excluding all extracranial injuries AIS ≥3. Patient demographics, clinical characteristics on admission, injury profile, Injury Severity Score, AIS, in-hospital morbidity, and beta-blocker exposure were abstracted for analysis. The primary outcome evaluated was in-hospital mortality stratified by pre-injury beta-blockade exposure.

**Results:** Overall, a total of 662 patients met study criteria. Of these 25% (n=159) were exposed to beta-blockade prior to their traumatic insult. When comparing the demographics and injury characteristics between the groups, the sole difference was age with the beta-blocked group being older (69 ± 12 yrs vs. 63 ± 13 yrs, $p$ <0.001). Beta-blocked patients had a higher rate of infectious complications (30% vs. 19%, $p$ = 0.04), with no difference in cardiac or pulmonary complications between the cohorts. Patients exposed to beta-blockade vs. no beta-blockade experienced 13% and 22% mortality, respectively ($p$ = 0.01). Stepwise logistic regression predicted the absence of betablockade exposure as a risk factor for mortality (OR 2.7, 95% CI 1.5-4.8, $p$ = 0.002). After adjustment for significant differences between the groups, patients not exposed to beta-blockade experienced 2- fold increased risk of mortality (AOR 2.2, 95% CI 1.3-3.7, $p$ = 0.004).

**Conclusion:** Pre-injury beta-blockade improves survival following isolated severe traumatic brain injury. The role of prophylactic beta-blockade and the timing of initiation of such therapy after traumatic brain injury warrant further investigations.

# A morphological study on the neurological defects of the pericyte deficient mouse in perspective of diabetic retinopathy

**Mollick T[1], Genové G[2], Johannson K[1]**

[1]School of Health and Medicine, Örebro University, Örebro, Sweden
[2]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

**Objective:** Diabetic Retinopathy (DR) is a neurovascular disorder characterized by microvascular lesions and neural circuitry abnormalities. Since pericyte deficiency has been linked as a primary cause in vascular dysfunction associated with DR, the *pdgf-b*$^{ret/ret}$ (platelet derived growth factor b retention motif knockout) mouse retina may replicate a similar pathological scenario. In this study we morphologically investigate the neurological defects of the *pdgf-b*$^{ret/ret}$ mouse retina and propose that it may be a suitable model for pathophysiological study of DR.

**Methods:** Immunofluorescence techniques were used to morphologically investigate the retinas collected from *pdgf-b*$^{ret/ret}$ mice at postnatal day (P)7, 10, 15 and 28.

**Results:** Vascular abnormalities were apparent from P10, however, prominent neuronal defects were mostly observed from P15, beginning with the compromised integrity of the laminated retinal structure characterized by the presence of rosettes and focally distorted regions. Photoreceptor degeneration was observed by loss of both rods and cones, including the altered structure of their synaptic terminals. Significant shortening of cone outer segments was observed from P10 and later stages; however, decrease in cone density was only observed at P28. Moreover, in response to retinal injuries, Müller and microglial cells were observed to be in the activated phenotype from P15 and onwards.

**Conclusions:** The *pdgf-b*$^{ret/ret}$ mouse retina displays a short time frame, between P10 and P15, during which retinopathic events and subsequent limitations in vision develop. This window may be critical for neuroprotective interventions in light of future research on experimental DR.

# Prognostic impact of the expression of Hedgehog proteins in cervical carcinoma FIGO stage I-IV treated with chemoradiotherapy

**Mordhorst Bohr L., Ahlin C., Sorbe B.**

Dept. of Oncology, Örebro University Hospital, SE-701 85 Örebro, Sweden

**Objective:** Hedgehog (Hh) signaling was assessed in patients with primary cervical carcinoma who were receiving chemoradiation. Because the up-regulation of Hh in carcinomas, has been reported, the authors examined associations between Hh and prognosis in cervical carcinoma.

**Methods:** In all, 131 cases of invasive cervical carcinoma were immunohistochemically analyzed for Patched (PTCH), smoothened (SMO), and glioma-associated oncogene family zinc finger 1 (Gli1), 2 (Gli2) and 3(Gli3) protein expression. Possible correlations between Hh expression, clinicopathologic data and the clinical outcome parameters were examined.

**Results:** Positive immunohistochemical staining for Hedgehog proteins was recorded in 21% to 46% of the tumor cells evaluated. The highest frequency was noted for SMO (46%) and the lowest for GLI1 (29%). The distribution of positive SMO cells was typically bimodal.
Tumors with overexpression of SMO were significantly ($P$ = 0.046) more often HPV-positive (92%) than tumors with low SMO staining (59%). There was a significant ($P$ = 0.042) association between low SMO-expression and KRAS-mutation. There was also a statistically highly significant ($P$ = 0.004) difference in KRAS-mutation frequency in tumors expressing GLI2 in 5-25% of the cells (27% mutation) compared with tumors expressing GLI2 in 26-50% of the cells (0% mutations). Tumors with overexpressed SMO had a higher frequency of tumor persistence or local recurrences (33.3%) than tumors with low SMO expression (6.5%). This difference was statistically significant ($P$ = 0.032). Patients with tumors expressing PTCH in more than 75% of the cells had significantly ($P$ = 0.023) better recurrence-free survival than patients with tumors expressing PTCH in less than 5% of the cells. The opposite situation was true for SMO expression, where tumors with low expression (< 5%) had a significantly ($P$ = 0.033) better prognosis than tumors with high expression (> 50%).
For GLI2 expression, there was a statistically significant difference with regard to overall ($P$ = 0.004) and distant ($P$ = 0.015) relapse rate for groups with expression of GLI2 in the range of 5-25% compared with 26-50%.

**Conclusions:** The Hedgehog signaling pathway seems to be of importance in cervical carcinoma as well as in the precursor lesions. In advanced invasive carcinomas treated with radiotherapy its role is probably less important and especially for distant tumor spread. Still, a predictive and prognostic value was found for PTCH, SMO, and GLI2 with regard to residual carcinoma after therapy, local recurrences and for GLI2 distant relapses. Thus, the Hedgehog signaling pathway seems to play an important role in cervical carcinogenesis together with HPV-infection and KRAS-mutation, and blockage of this pathway may be a potential treatment option in the future

**References:**

1. *Chaudary et al. Hedgehog pathway signaling in cervical carcinoma and outcome after chemoradiation. Cancer 2012; 118: 3105-3115.*
2. *Liu et al. Study of hedgehog signaling in cervical cancer. Proc Amer Assoc Cancer Res 2005; 46: Abs. 191.*

# Proteomic analysis of fibroblasts from chronic wounds and its relation to in vitro cellular ageing

**Ngwe Stella[1], Daniel Bergemalm[2], Fotabe Leslie[2] and Mikael Ivarsson[1]**

[1]Dept of Clinical Medicine, School of Health and Medical Science, Örebro University Örebro Sweden
[2]Dept of Medicine, Örebro University Hospital, Örebro, Sweden.

**Objective:** Over the past decade, chronic wounds have replaced other diseases in the elderly such as lung and heart diseases. Thus chronic wounds pose a great healthcare challenge. Fibroblasts are pivotal to the aberrant healing of the disease. In this study we examined the protein profile of cultured fibroblasts from chronic wounds as well as from early and late passage normal fibroblasts to investigate if cellular ageing reflects disease phenotype. The results show different protein patterns between normal and disease fibroblasts. Cellular ageing could in part recapitulate disease phenotype. Also growth abilities of the fibroblasts were studied. For the comprehension of the role of cellular ageing in the ulcers we also compared normal early and late passage senescent cells to simulate ageing.

**Methods:** Tissue biopsies of about $2mm^3$ were collected from leg ulcers and normal skin of six patients attending the Dermatology Department at Örebro University Hospital. Chronic wound fibroblasts (CWF) and normal fibroblasts (NF) were established in cultures. In addition, three fibroblasts primary cultures from normal skin biopsies were driven to senescence in culture. Proliferation rate between early and late passage of the fibroblasts were monitored by metabolic analysis. This was also performed on the 3 pairs of NF and CWF. For proteomics, two-dimensional gel electrophoresis was used for the separation of proteins.

**Results.** Senescent fibroblasts and CWF showed a decreased tendency to proliferate with respect to their early and normal counterparts. Analysis of results from 2D gel electrophoresis showed differentially identified proteins between NF and CWF, and also between early and later passages fibroblasts from normal skin.

**Conclusion:** Proteins identified in NF but absent in CWF are currently being identified. These could guide in a better understanding of the disease. In particular, leg ulcers inability to completely heal could be a result of cellular ageing of the fibroblasts, which do not provide cues necessary for wound healing. This scenario could be improved by e.g. introducing lacking growth factors to the ulcers for complete healing.

# Effects of vitamin C on pro-inflammatory cytokine induced oxidative stress and tyrosine uptake in fibroblasts

**Nordmark J.[1,2], Vumma R.[2], Venizelos N.[2]**

[1] School of Medicine and [2]School of Health and Medical Sciences, Dept. of Clinical Medicine, Örebro University, Örebro, Sweden

**Introduction:** Human dermal fibroblasts possess the same main transporter LAT1 (for dopamine precursor tyrosine) as found on blood-brain barrier micro endothelium, and fibroblasts show disturbed tyrosine transport in several neuropsychiatric disorders. Inflammation and oxidative stress have gained interest as targets for biomarkers of neuropsychiatric disorders. Moreover, adjuvant therapies with anti-inflammatory substances and antioxidants have been used in clinical studies. This study examines if tyrosine uptake is affected by pro-inflammatory cytokine induced radical oxygen species (ROS) formation in human dermal fibroblasts, and effects of antioxidant Vitamin C.

**Methods:** A single cell line of human dermal fibroblasts was treated for 3 h with combinations of cytokines (IL-1β or IL-6 in combination with TNFα and IFNγ) either with or without the supplement of Vitamin C. ROS was measured as fluorescent response after treatment with 2', 7'-dichlorodihydrofluorescein diacetate ($H_2$DCFDA). Cells were examined for uptake of [L-$^{14}$C]-tyrosine using the cluster tray method. Experiments were performed in triplicates.

**Results:** Cytokine induced cellular ROS was inversely associated with uptake of tyrosine when compared to control, most clearly seen for IL1β-combination where 12 % increase in fluorescence was associated with 33 % decrease in uptake of tyrosine. Vitamin C supplemented to IL6-combination counteracted the negative effect of these cytokines on tyrosine uptake, compared to control.
**Conclusions:** Early inflammatory processes cause oxidative stress and subsequent decrease in tyrosine uptake, Vitamin C partially or completely restore the latter. These findings could point towards ROS potentially damaging the amino acid transporters and/or surrounding plasma membrane, thus lowering transmembrane transport of tyrosine.

**References:**

1. *Vumma R, Wiesel F-A, Flyckt L, Bjerkenstedt L, Venizelos N. Functional characterization of tyrosine transport in fibroblast cells from healthy controls. Neurosci. Lett. 2008;434:56–60.*
2. *Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M et al. Pathways underlying neuroprogression in bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience and Biobehavioral Reviews 2011:35;804–817.*

# Subjective well-being and its relation to physical activity in Swedish active seniors

**Olsson LA [1,2], Hurtig-Wennlof A [1], Nilsson TK [2]**

[1]Orebro University, School of Health and Medical Sciences, Örebro, Sweden
[2] Orebro University Hospital, Dept of Laboratory Medicine, Örebro, Sweden

**Objective**: Well-being (WB) is a complex variable in its relation between health and other personal and social characteristics. Physical activity (PA) is often claimed to be related to WB. Another aspect of WB is Subjective wellbeing (SWB) the person's own evaluation of his or her life. The aim was to study possible associations of SWB with selected biomarkers of cardiovascular risk and physical activity, in a sample of Swedish active seniors.

**Methods**: The sample consisted of 389 community dwelling senior citizens recruited from several retired persons' organizations. Serum samples were analysed for lipoproteins and makers of inflammation.
The Psychological General Well-Being (PGWB) index was used to measure subjective well-being or distress during the past week. PA was assessed by a version of the International Physical Activity Questionnaire modified for elderly (IPAQ-E )

**Results:** The distribution of PA categories in the sample was: *Low*=15%; *Moderate*=32%; and *High* 53%, and no gender differences were observed. Of the PGWB sub-dimensions, General Health had the strongest relation with PA while sex, age and biomarkers of somatic health had a minor contribution to the variance.

**Conclusion:** IPAQ-E is an useful instrument for assessing physical activity.
Physical activity is positively related to self-reported subjective well-being, and account to a higher degree than biomarkers of cardiovascular risk to the variance in SWB in this cohort of active Swedish seniors

# *Lactobacillus rhamnosus* strains modulate *Escherichia coli* induced CXCL8 and IL-6 secretion in macrophage-like cells

**Thomas Roxlau[1,2], Anna Lindblad[2], Robert J Brummer[2], JanaJass[1]**

[1]School of Scienceand Technology, The Life Science Center, and
[2]School of Health and Medical Science, Nutrition Gut Brain Interactions, Örebro University

**Background:** Probiotic lactobacilli can modulate the gut microbiome diversity [1] and regulate immune response s [2].We have shown that macrophage-like cells respond to the probiotic *Lactobacillus rhamnosus* GR-1and thus providing evidence for regulatory mechanisms that intervene with CXCL8 and IL-6 processing [2,3].The aim of this study is to investigate the immunomodulatory effects of known probiotic and a non-probiotic *L. rhamnosus* strains on macrophage-like cells.

**Methods:** Macrophage-differentiated THP-1 cells were co-stimulated with *L. rhamnosus* GG (intestinal probiotic), GR-1 (urogenital probiotic) or type strain ATCC7469 (non-probiotic) together with heat-killed (hk-) *Escherichia coli.* The temporal intraandextracellularCXCL8 and IL-6 levels were evaluated by enzyme-linked immunosorbent assay (ELISA).

**Results:** The induction of an inflammatory response in macrophage-like cells by stimulation with hk- *E. coli* resulted in increasing levels of intra and extracellular CXCL8 and IL-6, however, co-stimulation with different *L. rhamnosus* strains (GG, GR-1, 7469) significantly ($p<0.05$) inhibited this response. The inhibitory effect on cells seemed to be inherent for all *L. rhamnosus* strains tested, with the strongest effect observed with the probiotic strains (*L. rhamnosus* GG>GR-1) and the weakest with the non-probiotic strain (ATCC 7469). Exposure of cells to probiotic strains alone showed no effect on the basal cytokine levels, while the non-probiotic strain triggered a weak basal immune response, compared to control cells.

**Conclusion:** The intracellular and extracellular levels of CXCL8 and IL-6 levels in response to treatment with hk- *E. coli*, together with probiotic or non-probiotic strains of *L. rhamnosus*, provided evidence that regulation of cytokine secretion takes place intracellularly.

**References:**

*[1] Cox MJ, Huang YJ, Fujimura KE, Liu JT, McKean M, Boushey HA, Segal MR, Brodie EL, Cabana MD, Lynch SV. Lactobacillus casei abundance is associated with profound shifts in the infant gut microbiome. PLoS One.2010 Jan 18; 5 (1):e 8745*

*[2] Karlsson M, Jass J. Lactobacilli differently regulate expression and secretion of CXCL8 in urothelial cells. Benef Microbes. 2012 Sep; 3(3):195-203.*

*[3] Abuabaid H, Karlsson M , Scherbak N, Olsson PE, Jass J. Probiotic Lactobacillus rhamnosus alters inflammatory responses of bladder epithelial and macrophage-like cells in co-culture (unpublished)*

# Patients' perceptions of the concept participation in forensic psychiatric care – a phenomenographic study

**Selvin M (1), Almqvist K (2), Kjellin L (1), Schröder A (1)**

1) Psychiatric Research Centre, Örebro County Council and School of Health and Medical Sciences, Örebro University, Sweden
2) Department for Research and Development, Psychiatry Division, Country Council of Värmland and Department for Psychology, Karlstad University

**Background:** Patient participation is a central concept in health care but in studies of quality in the psychiatric care the patients often rate questions about participation low. The concept of participation is multidimensional and complex and the meaning can depend on the context. The literature describes the concept in different ways and very few describe it from the patients' perspective. There are some studies of the concept from the patients' perspective in somatic care but it is not necessarily comparable with psychiatric care. The question about participation is especially complex in coercive care. There are no earlier studies published about patients' perceptions of the concept within forensic psychiatry.

**Objective:** The aim of this study is to describe patients' perceptions of the concept of participation in forensic psychiatric care.

**Design:** It is a qualitative interview study with 20 patients in forensic psychiatric care.

**Methods:** A phenomenographic approach was used in the study.

**Results:** Preliminary results of the ongoing analysis show that patients in forensic psychiatry are able to give a rich and varied description of the concept.

**Relevance:** This study is expected to give a deeper understanding of how patients in forensic care perceive the concept of participation and to give decision makers and staff important knowledge in the constant process of developing the forensic psychiatric care.

# The changing role of the Swedish elite football coach

**Svensson Robert**

The institution of health and medicine, Örebro University, Örebro, Sweden

**Objective:** The coach has become a leading figure in Swedish elite football. Simultaneously as professionalization, commercialization, medialization, scientification and the increase organization of the game have shaped football in general; these processes have placed the coach in its absolute center. They have more responsibility and power; however there are now more factors to deal with. The aim of this study is to get a deeper understanding of this development. The year of departure is 1967 when the amateur regulations were overturned and the processes above intensified.

**Methods:** Text analysis of the educational material from the coaching course organized by the Swedish football association (SvFF) and of documents from elite football clubs, as well as qualitative interviews with current and former players, coaches and leading person in Swedish elite football, will be used to examine why and how the role of the coach has changed. The theoretical framework is a governmentality perspective and the analyze tools are two settings of ideal types.

**Results:** A preliminary result coming from the analysis of the first half of the educational material shows that the range of knowledge that a coach needs, is now broader and deeper. Apart from practical football skills; psychological, physiological, pedagogical and tactical aspects have become more prominent.

**Conclusions:** These findings highlight how the roles and responsibilities of the coach has increased, from an instructor to a manager, mentor and also, to a greater extent, coaching the game.

# Impaired metabolic control and socio-demographic status in immigrant children at onset of type 1 diabetes

**Ulf Söderström MD[1 2 3], Ulf Samuelsson MD PhD[5 6], Lotta Sahlqvist[2], Jan Åman MD PhD[1 4]**

[1] School of Health and Medical Sciences Örebro University, Sweden
[2] Centre for Clinical Research Sörmland, Uppsala University, Sweden;
[3] Department of Pediatrics, Mälarsjukhuset Hospital, Eskilstuna, Sweden,
[4] Department of Pediatrics Örebro University Hospital, Sweden
[5]Division of Pediatrics, Department of Molecular and Clinical Medicine, Linkoping University, Sweden
[6]Department of Pediatrics, the University Hospital in Linköping, Sweden

**Aims/hypothesis:** The aim of the present study was to compare clinical and socio-demographic conditions at disease onset of type 1 diabetes in children born to immigrant families and children born to Swedish families, and to assess whether those conditions had an impact on metabolic status.
Design: Observational nationwide population-based matched cohort-study on prospectively recorded registry data.
Setting: All children with diabetes in Sweden and their families during 2000 – 2010.
Patients: 879 children with diabetes born to immigrant parents out of a total of 13,415 diabetic children were assigned to the cases. To these we added a control group of 2,627 children with Swedish-born parents, matched for gender, age and year of onset

**Results:** The proportion of low capillary pH (< 7.30) was higher in the 879 immigrant children, 25.8 %, than in the controls, 16.4 % (p = 0.000). $HbA_{1c}$ was higher, 95 mmol/mol (10.8 %) and 88 (10.2), respectively (p = 0.000).
We used a logistic regression model for low pH at disease onset and tested the impact of clinical and socio-demographic factors. Whereas we were unable to reveal any significant influence for socio-demographic parameters, metabolic parameters displayed significance.

**Conclusion:** Children born to immigrant parents have lower capillary pH and higher $HbA_{1c}$ at diabetes onset. Immigrant families have lower socio-demographic living conditions, but this fact does not seem to influence the inferior metabolic start at diabetes onset.

***Key words:*** diabetes type 1, $HbA_{1c}$, children, adolescents, epidemiology, ethnology, immigration

# The development of an assessment scale for measuring care dyads' person transfer related behavior in dementia settings

**Thunborg, C[1] [2],von Heideken Wågert, P[1], Ivarsson, A-B[2] , Söderlund, A[1]**

[1]School of Health, Care and Social Welfare, Mälardalen University, Västerås, Sweden
[2]School of Health and Medical Sciences, Örebro University, Örebro, Sweden

**Objective:** Suffering from dementia means restricted motor behavior due to decreased motor control, visuo-spatial working memory, and executive functioning. Person transfer situations are important daily activities in dementia settings and care dyads (CD) reciprocally struggles to find right solutions for their performance. The complexity of restricted motor behavior in the person with dementia makes dyadic interaction in these person transfer situation highly unpredictable. There is no assessment scale taking into account factors related to CDs interactional behavior in person transfer situations. This project aimed at develops a new assessment scale for CD interaction in person transfer situations.

**Method:** First a conceptual framework for the concept Reciprocal Struggling (RS) was developed. For finding important attributes for RS a literature review was performed. To describe the attributes and for further item generation video recorded person transfer situations in dementia settings were gathered. In the next step an item pool was created and 15 registered physiotherapists were asked to rate the importance of 92 proposed items on a four point Liker-type scale.

**Results:** The literature review, video observations and physiotherapists' prioritization resulted in an assessment scale containing seventeen items in two areas, (a) the person with dementia and (b) the professional caregiver. The assessment scale should identify bio-psychosocial factors that can be intervened in problematic person transfer situations in dementia settings.

**Conclusion:** In conclusion the assessment scale can have importance for identifying factors that can be intervened in problematic person transfer situations in dementia settings.

# Effect on left ventricular mass and geometry in patients with takotsubo cardiomyopathy

**Waldenborg M[1*], Lidén M[2], Thunberg P[3], Emilsson K[1]**

[1]Department of Clinical Physiology, Örebro University Hospital, Sweden ([*]School of Health and Medical Sciences, Örebro University).
[2]Department of Radiology, Örebro University Hospital, Sweden.
[3]Department of Biomedical Engineering, Örebro University Hospital, Sweden.

**Objective:** Takotsubo cardiomyopathy (TTC) is a condition of reversible left ventricular (LV) dysfunction. In a previous magnetic resonance imaging (MRI) study, changes in LV mass and geometry were documented at TTC (1). Transthoracic echocardiography (TTE) is known as a more easily accessible technology. We performed a retrospective analysis of our cohort of TTC patients, to further investigate these LV changes, including comparison of TTE and MRI.

**Methods:** We studied 13 female TTC-patients. All had TTE and MRI examinations at onset, and 3 months later at follow-up. LV mass was assessed with MRI and 5 different TTE-methods. Segmental wall thickness (SWT) was measured, and used for LV geometry classification according to guidelines (2). Radial strain (%) was measured at TTE. Differences were analyzed with Wilcoxon Signed test and McNemar's test. Spearman's coefficient was used for intertechnique concert (with Bland Altman plot), and for correlations of simultaneous TTE changes between phases.

**Results:** LV mass was significantly decreased between phases ($p<0.05$), by MRI and with 2 TTE-methods. Two of 3 SWT points were significantly decreased, with adequate correlation ($r>0.70$) between MRI and TTE, while geometry categories remained unchanged. One TTE LV mass-method (truncated ellipsoid) showed clearly better consistency towards MRI. Radial strain was significantly improved with adequate correlation towards the truncated ellipsoid method.

**Conclusions:** TTC is associated with acute increase in LV mass, which seems to be a local effect. This effect parallels with improvement in concentric LV wall motion. MRI and TTE show adequate consistency, primarily for truncated ellipsoid regarding LV mass.

**References**

1. *Stensaeth KH, Fossum E, Hoffmann P, Mangschau A, Skretteberg PT, Klow NE. Takotsubo cardiomyopathy in acute coronary syndrome; clinical features and contribution of cardiac magnetic resonance during the acute and convalescent phase.* ***Scand Cardiovasc J 2011;45(2):77-85.***
2. *Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification.* ***Eur J Echocardiogr 2006;7(2):79-108.***

# Title: Characterization of Zinc finger protein496 (Znf496) in human umbilical vein endothelial cells (HUVECs)

**Wu R, Zhang B, Sirsjö A, Fransén K**

School of Health and Medical Sciences, Örebro University, Örebro, Sweden

**Introduction:** Znf496 is one of the members in Zinc finger protein family, which functions haven't been clarified yet. Previous data indicated on an association between Znf496 and atherosclerosis, since a pilot study showed that plaque tissue was positive staining for the Znf496 protein in the endothelial layer. To explore the potential role for Znf496, we have studied the impact of Znf496 on cell proliferation, migration tube formation and downstream target genes in HUVECs.

**Methods:** HUVECs were treated with siRNA against Znf496 for 24h to 72h. After knock-down, real-time PCR and Western blot were performed to confirm the efficiency on knock down samples on mRNA and protein levels. Cell proliferation, wound recovery and tube formation assays were performed to verify knock-down effects on the cells. Microarray on Znf496 knock-down and wild type cells was performed and the results were verified with real time PCR.

**Results:** While knock-down Znf496 in HUVECs, the cell proliferation, wound recovery and tube formation assays showed HUVECs basic functions were altered, such as inhibition effects in proliferation, wound recovery and tube formation assays. Microarray data showed significantly altered expression of 161 genes due to the Znf496 knock-down process. Among the down-regulated genes, the cell cycle genes and NOD- like receptor pathway genes were present.

**Conclusion:** Znf496 is involved in cell growth, tube formation and migration in the HUVECs and the array data suggests several potential interesting target genes in the field of cell cycle and inflammation.

# *Porphyromonas gingivalis* infection is associated with the expression of Angiopoietin-1 and Angiopoietin-2 in Aortic Smooth Muscle Cells

**Zhang B, Khalaf H, Sirsjö A and Bengtsson T.**

Department of Clinical Medicine, Örebro University, Örebro, Sweden

**Background:** *Porphyromonas gingivalis* is a gram-negative bacterium that is involved in the development of cardiovascular disease. Angiopoietin 1 (angpt1) and angiopoietin 2 (angpt2) are the ligands for Tie receptors and play important roles for vessel development and the development of inflammatory diseases, such as atherosclerosis. The aim of this study was to investigate the effects of *P. gingivalis* infection on gene and protein expression of angpt1 and angpt2 and their relation to cellular function.

**Methods:** AoSMCs were exposed to different mutant of viable *P. gingivalis* for 24h, whereafter quantitative real-time PCR was used to study gene expression of angpt1 and angpt2. Immunocytochemestry was applied to investigate the protein expression of angpt2. Reactive oxygen species (ROS) production was measured by DCFDA method. The role of angpt2 on cell migration was checked by scratch assay.

**Results:** We found that the wide type *P. gingivalis* ATCC and W50 are significantly increasing the gene expression of angpt2, so as the protein level in AoSMCs. The angpt1 gene expression was reduced by *P. gingivalis*. Nevertheless, the rgpA and rgpB double mutant E8 has no effect on the gene and protein expression of angpt1 and angpt2 in AoSMCs. E8 also induce low level of ROS production compared with wide type strains. Angpt2 protein was revealed to enhance the migration of AoSMCs.

**Conclusions:** This study demonstrates that *P. gingivalis* is able to affect angiopoietins production in AoSMCs which are involved in the development of athrosclerosis. These findings further support the association between periodontitis and cardiovascular diseases.

# Author Index, Nobel Day 10 of December 2013


| | Abstract/Poster Page nr |
|---|---|
| Samal Algilani, Lina Östlund-Lagerström, Annica Kihlgren1, Robert J. Brummer, Ida Schoultz | **1** |
| Vladimir T. Basic, Annette Jacobsen, Allan Sirsjö and Samy M. Abdel-Halim | **2** |
| Vladimir T. Basic, Annette Jacobsen | **3** |
| Vladimir T. Basic, Elsa Tadele, Annette Jacobsen, Allan Sirsjö1 and Samy M. Abdel-Halim | **4** |
| Bergh C, Udumyan R, Fall K, Nilsagård Y, Appelros P, Montgomery S | **5** |
| M. Ewertsson, R. Allvin2, I.K. Holmström3, K. Blomberg1 | **6** |
| Sanja A Farkas, Nina Milutin-Gašperov, Magdalena Grce, and Torbjörn K Nilsson | **7** |
| Fotabe L, Kruse R , Åström M, and Ivarsson M | **8** |
| Fälker K, Ljungberg L, Grenegård M | **9** |
| Ganda Mall JP, Östlund-Lagerström L, Löfvendahl L, Keita AV, Brummer RJ1, Schoultz I | **10** |
| George Gkoumas1, Geena Paramel Varghese, Karin fransén, Allan Sirsjö | **11** |
| Sezin Günaltay, Nils Nyhlin , Ashok Kumar Kumawat, Curt Tysk, Johan Bohr, Olof Hultgren, and Elisabeth Hultgren Hörnquist | **12** |
| Sezin Günaltay, Nils Nyhlin, Ashok Kumar Kumawat, Curt Tysk, Johan Bohr, Olof Hultgren, and Elisabeth Hultgren Hörnquist | **13** |
| Shahida Hussain, Godfried M. Roomans and Eva Särndahl | **14** |
| Berhane Asfaw Idosa, Berolla Sahdo, Ermias Balcha, Anne Kelly, Bo Söderquist, Eva Särndahl | **15** |

# Author Index, Nobel Day 10 of December 2013


| | Abstract/Poster Page nr |
|---|---|
| Jayaprakash Kartheyaene, Khalaf Hazem, Sirsjö Allan, Bengtsson Torbjörn | **16** |
| Koskela Anita och Ivarsson Mikael. | **17** |
| Kumakech E, Achora S; Berggren V, Bajunire F. | **18** |
| Kumakech E, Anderson S, Wabinga H, Berggren V | **19** |
| Madelene Larsson, Camilla Pettersson, Therése Skoog, Charli Eriksson | **20** |
| Lillsunde Larsson G, Helenius G 1, Andersson S, Sorbe B, Karlsson M 1 | **21** |
| Helenius G, Lillsunde-Larsson G, Andersson S, Unemo M, Karlsson MG | **22** |
| M Lindh, A Kihlgren, K-I Perseius | **23** |
| Logotheti M, Pilalis E., Shemirani I.M., Chatziioannou A., Venizelos N., Kolisis F. | **24** |
| LuhrK, Marie Holmefur, Ulrica Nilsson, Ann Catrine Eldh | **25** |
| Malm K, Unemo M, Thulin Hedberg S, Kireev D, and Andersson S | **26** |
| Adrian Meehan, Mats Humble, Payam Yazarloo, Johannes Järhult, Göran Wallir | **27** |
| Shahin Mohseni, Peep Talving, Göran Wallin, Olle Ljungqvist, Louis Riddez | **28** |
| Mollick T, Genové G, Johannson K | **29** |
| Mordhorst Bohr L. Ahlin C. Sorbe B. | **30** |

# Author Index, Nobel Day 10 of December 2013


| | Abstract/Poster Page nr |
|---|---|
| Ngwe Stella, Daniel Bergemalm, Fotabe Leslie and Mikael Ivarsson | **31** |
| Nordmark J., Vumma R., Venizelos N. | **32** |
| Olsson LA, Hurtig-Wennlof A, Nilsson TK | **33** |
| Thomas Roxlau1, Anna Lindblad, Robert J Brummer, Jana Jass | **34** |
| Selvin M, Almqvist K, Kjellin L, Schröder A | **35** |
| Svensson Robert | **36** |
| Ulf Söderström MD, Ulf Samuelsson MD PhD, Lotta Sahlqvist, Jan Åman MD PhD | **37** |
| Thunborg, C,von Heideken Wågert, P, Ivarsson, A-B, Söderlund, A | **38** |
| Waldenborg M*, Lidén M, Thunberg P, Emilsson K | **39** |
| Wu R, Zhang B, Sirsjö A, Fransén K | **40** |
| Zhang B, Khalaf H, Sirsjö A and Bengtsson T. | **41** |


Lovely, we don't need to rent a frack for the Nobel Day Festivities