

## CROATIAN INTERNATIONAL PUBLICATIONS

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**Lauc G, Essafi A, Huffman JE, Hayward C, Knežević A, Kattla JJ et al. Genomics meets glycomics—the first GWAS study of human N-glycome identifies HNF1α as a master regulator of plasma protein fucosylation. *PLoS Genet.* 2010;6:e1001256.**

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Over half of all proteins are glycosylated, and alterations in glycosylation have been observed in numerous physiological and pathological processes. Attached glycans significantly affect protein function; but, contrary to polypeptides, they are not directly encoded by genes, and the complex processes that regulate their assembly are poorly understood. A novel approach combining genome-wide association and high-throughput glycomics analysis of 2,705 individuals in three population cohorts showed that common variants in the Hepatocyte Nuclear Factor 1α (HNF1α) and fucosyltransferase genes FUT6 and FUT8 influence N-glycan levels in human plasma. We show that HNF1α and its downstream target HNF4α regulate the expression of key fucosyltransferase and fucose biosynthesis genes. Moreover, we show that HNF1α is both necessary and sufficient to drive the expression of these genes in hepatic cells. These results reveal a new role for HNF1α as a master transcriptional regulator of multiple stages in the fucosylation process. This mechanism has implications for the regulation of immunity, embryonic development, and protein folding, as well as for our understanding of the molecular mechanisms underlying cancer, coronary heart disease, and metabolic and inflammatory disorders.

**Bašić-Jukić N, Hudolin T, Radić-Antolić M, Čorić M, Zadro R, Ž, Pasini J et al. Bone morphogenetic protein-7 expression is down-regulated in human clear cell renal carcinoma. *J Nephrol.* 2011;24:91-7.**

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Background: Recent studies demonstrated that the expression pattern of bone morphogenetic protein-7

(BMP-7) is altered in different tumors. We determined expression of BMP-7 in human clear cell renal carcinoma (CCRC). Methods: Samples from cancer and corresponding healthy tissue were obtained from 20 patients who underwent nephrectomy for CCRC. Expression of BMP-7 mRNA was determined by reverse transcriptase polymerase chain reaction (RT-PCR), and protein expression was analyzed by immunohistochemistry. Results: RT-PCR showed strong down-regulation of BMP-7 mRNA in cancer tissue. Immunohistochemistry revealed expression of BMP-7 in normal renal tissue, with almost complete loss of BMP-7 expression in malignant cells of 6 patients (30%). After 3 years of follow-up, 5 out of 6 patients with high BMP-7 mRNA expression were alive and disease-free, compared with 9 out of 14 patients with low BMP-7 mRNA expression. Conclusions: BMP-7 mRNA and protein expression were down-regulated in CCRC. Further prospective studies are needed to characterize the role of BMP-7 in human CCRC.

**Cepika AM, Bendelja K, Vergles JM, Malenica B, Kapitanović S, Gagro A. Monocyte response to LPS after exposure to corticosteroids and chloroquine with implications for systemic lupus erythematosus. *Scand J Immunol.* 2010;72:434-43.**

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Essential part of a response to infection is early pathogen recognition and adequate initiation of innate immunity. One of the hallmarks of systemic lupus erythematosus (SLE) is reduced resistance to infection despite overall hyperactivity of the immune system. Immunosuppressive drugs (high-dose corticosteroids and cytotoxic agents) are independent risk factors for infection in SLE, with bacteria as predominant cause. To investigate whether less aggressive immunomodulatory treatment may still affect recognition and response to Gram-negative bacteria, we measured TLR4 expression in monocytes of untreated SLE patients and patients on chloroquine and low-dose steroid therapy and examined the drugs' influence on monocyte TLR4 expression in peripheral blood mononuclear cell (PBMC)

culture. Additionally, we determined whether induction of monocyte NF- $\kappa$ B signalling, TNF- $\alpha$  and IL-6 production with lipopolysaccharide (LPS), a TLR4 ligand, can be altered with dexamethasone, chloroquine or both. There was no statistically significant difference in TLR4 expression between patients with SLE and controls, even though treated SLE patients tended to have lower frequency of TLR4(+) monocytes and TLR4 mean fluorescence intensity than healthy controls. However, neither dexamethasone nor chloroquine had major influence on TLR4 expression in vitro or suppressed LPS-induced NF- $\kappa$ B activation in monocytes, although dexamethasone decreased TNF- $\alpha$  and IL-6 production. Therefore, even if low-dose steroids or chloroquine do not seem to affect TLR4 expression and signalling, steroids might decrease cytokine production in response to LPS.

**Jani A, Zimmerman M, Martin J, Lu L, Turkmen K, Ravichandran K, Pačić A\*, Ljubanović D\*, Edelstein CL. Perfusion storage reduces apoptosis in a porcine kidney model of donation after cardiac death. *Transplantation*. 2011;91:169-75.**

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**BACKGROUND:** Donation after cardiac death (DCD) kidneys suffer a high incidence of delayed graft function attributable to warm ischemia and cold ischemia (CI). Neither the mechanism of injury nor type of cell death has been described. Clinical studies suggest that perfusion storage (PS) of DCD kidneys may reduce injury although the mechanism of protection is unknown. In a porcine model of DCD, we hypothesized that DCD kidneys have increased caspase-1 due to warm ischemia (WI) and increased caspase-3 and apoptosis due to CI. **METHODS:** Male Yorkshire pigs subjected to cardiac death were perfused with cold University of Wisconsin solution. The perfused kidneys were removed and stored in cold University of Wisconsin solution for 24 hr. Kidney biopsies were obtained before cardiac death and at 0 and 24 hr of CI. **RESULTS:** There was an increase in caspase-1 activity due to WI before cold preservation. CI was associated with a massive increase in apoptosis, caspase-3/7 activity, and caspase-3 protein expression. Next, we hypothesized that PS would protect against apoptosis. We compared DCD kidneys subjected to static versus PS for 24 hr. PS significantly reduced proximal tubular apoptosis and was associated with increased B-cell lymphoma-extra large, and hypoxia-inducible transcription factor-1 $\alpha$ . **CONCLUSIONS:** These findings suggest that in DCD kidneys, WI preferentially activates caspase-1, whereas CI activates caspase-3 and causes apoptosis. PS may protect DCD kidneys through activation

of antiapoptotic pathways involving B-cell lymphoma-extra large and hypoxia-inducible transcription factor-1 $\alpha$ .

**Antica M, Paradžik M, Novak S, Džebro S, Dominis M. Gene expression in formalin-fixed paraffin-embedded lymph nodes. *J Immunol Methods*. 2010;359:42-6.**

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Elucidation of molecular pathways involved in development of human lymphoma requires efficient methods for tackling gene expression in lymph nodes. Expression studies of transcription factors in these malignancies facilitate understanding the changes occurring in neoplastic transformation and lymphoma development. Excised lymph nodes are routinely fixed in formalin and embedded in paraffin for diagnosis and stored in many hospitals' pathology archives. These tissues represent a precious resource for research since they allow retrospective studies to cover a broad range of human lymphoma even the less frequent types. Reverse transcription polymerase chain reaction (RT-PCR) is a commonly used method for gene expression analysis and a reproducible protocol for RNA isolation from lymph nodes is an inevitable requirement for these studies. However, formalin fixation and paraffin-embedding interfere with the quality of RNA especially when isolated from lymph nodes being the most fragile lymphatic tissues. We present here a simple and fast method for RNA isolation from formalin-fixed paraffin-embedded lymph nodes that can be successfully applied for RT-PCR as well as for quantitative RT-PCR analysis. We tested diverse isolation reagents and combined a range of factors in order to get a high quality RNA for retrospective studies of gene expression in human lymphoma samples. Our modified method of RNA extraction from FFPE provides superior yields and purity based on qPCR data.

**Škrčić A, Korać P, Krišto DR, Ajduković Stojisavljević R, Ivanković D, Dominis M. Immunohistochemical analysis of NOTCH1 and JAGGED1 expression in multiple myeloma and monoclonal gammopathy of undetermined significance. *Hum Pathol*. 2010;41:1702-10.**

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Notch signaling is implicated in the pathogenesis of multiple myeloma expressing high level of active

Notch proteins NOTCH1 and JAGGED1 in tumor plasma cells. We investigated expression of NOTCH1 and JAGGED1 in bone marrow trephine biopsies of 80 newly diagnosed multiple myeloma and 20 monoclonal gammopathy of undetermined significance patients using immunohistochemical methods. The number of positive tumor cells was counted per 1000 tumor cells and the intensity of staining was assessed semi quantitatively. Multiple myelomas expressed NOTCH1 in 92.31% (72/78) and JAGGED1 in 92.21% (71/77) cases. NOTCH1 staining was strong in the majority of cases (59.7%), whereas JAGGED1 was predominately weak (67.6% of cases). In contrast, both markers were negative in all monoclonal gammopathy of undetermined significance cases. However, upon progression of disease from monoclonal gammopathy of undetermined significance to multiple myeloma (seen in 4 patients), analysis of the subsequent bone marrow biopsy showed weak expression of both markers in tumorous plasma cells. Immunohistochemistry results were compared with the pattern of bone marrow infiltration, plasma cell differentiation, and the presence of t(11;14)(q13;q32), t(14;16)(q32;q23), and t(4;14)(p16.3;q23) and overall survival in multiple myeloma patients. A significant correlation was found between strong NOTCH1 staining in multiple myeloma plasma cells and the diffuse type of bone marrow infiltration ( $P = .002$ ) and an immature morphologic type of plasma cells ( $P = .043$ ). After a median follow-up of 20.3 months, in multiple myeloma patients no difference in overall survival between NOTCH1 ( $P = .484$ ) and JAGGED1 ( $P = .822$ ) positive and negative cases were found. In conclusion, our results indicate importance of NOTCH1 and JAGGED1 expression in plasma cell neoplasia and a possible diagnostic value of their immunohistochemical evaluation of bone marrow infiltrates for multiple myeloma.

**Džamonja G, Tank J, Heusser K, Palada I, Valić Z, Baković D et al. Glossopharyngeal insufflation induces cardioinhibitory syncope in apnea divers. Clin Auton Res. 2010;20:381-4.**

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Apnea divers increase intrathoracic pressure voluntarily by taking a deep breath followed by glossopharyngeal insufflation. Because apnea divers sometimes experience hypotension and syncope during the maneuver, they may serve as a model to study the mechanisms of syncope. We recorded changes in hemodynamics and sympathetic vasomotor tone with microneurography during breath

holding with glossopharyngeal insufflation. Five men became hypotensive and fainted during breath holding with glossopharyngeal insufflation within the first minute. In four divers, heart rate dropped suddenly to a minimum of  $38 \pm 4$  beats/min. Therefore, cardioinhibitory syncope was more common than low cardiac output syncope.

**Karanović N, Pecotić R, Valić M, Jerončić A, Carev M, Karanović S et al. The acute hypoxic ventilatory response under halothane, isoflurane, and sevoflurane anaesthesia in rats. Anaesthesia. 2010;65:227-34.**

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The relative order of potency of anaesthetic agents on the hypoxic ventilatory response has been tested in humans, but animal data are sparse. We examined the effects of 1.4, 1.6, 1.8, and 2.0 MAC halothane, isoflurane, and sevoflurane on phrenic nerve activity in euoxia (baseline) and during acute normocapnic hypoxia (inspired oxygen fraction 0.09) in adult male Sprague-Dawley rats. With halothane, all animals became apnoeic even in euoxia, and the hypoxic response was completely abolished at all anaesthetic levels. With isoflurane, 5 of 14 animals exhibited phrenic nerve activity in euoxia at 1.4 MAC and demonstrated a hypoxic response (302% of baseline activity), but all became apnoeic and lost the hypoxic response at higher doses. With sevoflurane, phrenic nerve activity and a hypoxic response was preserved in at least some animals at all doses (i.e. even the highest dose of 2.0 MAC). Similar to the rank order of potency previously observed in humans, the relative order of potency of depression of the hypoxic ventilatory response in rats was halothane (most depressive) > isoflurane > sevoflurane ( $p = 0.01$  for differences between agents).

**Jovanović N, Božina N, Lovrić M, Medved V, Jakovljević M, Peleš AM. The role of CYP2D6 and ABCB1 pharmacogenetics in drug-naïve patients with first-episode schizophrenia treated with risperidone. Eur J Clin Pharmacol. 2010;66:1109-17.**

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**PURPOSE:** To evaluate the role of cytochrome 450 2D6 (CYP2D6) and ABCB1 variants on plasma risperidone concentrations and treatment response in 83 drug-naïve patients experiencing a first episode of psychosis. **METHODS:**

All patients were treated with risperidone for 8 weeks. The CYP2D6 genotyping was performed by allele-specific PCR-restriction fragment length polymorphism analysis (for alleles \*3,\*4,\*6) and long-distance PCR (for duplications and allele \*5), while real-time PCR analysis was used for the ABCB1 G2677T/A and C3435T variants. Plasma concentrations of risperidone and 9-OH risperidone were measured by high-performance liquid chromatography. RESULTS: The number of patients with the CYP2D6 wild type (wt)/wt, wt/mutation (mut) and mut/mut genotype was 43, 32 and 8, respectively. The number of patients with the ABCB1 2677G/G, G/T and T/T variants was 29, 42 and 12, respectively; those with the 3435CC, C/T and T/T variants was 25, 37 and 21, respectively. The CYP2D6 genotype had a strong effect on the steady-state dose-corrected plasma levels (C/D) of risperidone, its 9-OH metabolite and the active moiety, while the ABCB1 2677 T/T and 3435 T/T genotypes has similarly strong effects on the active moiety C/D. The CYP2D6 poor metabolizers had a significantly higher risperidone C/D and active moiety C/D and lower 9-OH risperidone C/D. The ABCB1 3435 T allele and the ABCB1 2667 T-3435 T haplotype carriers were more frequent among subjects without extrapyramidal syndromes. Patients showed significant improvements in positive and general symptoms, but not in negative symptoms. These changes were not related to variations in genetic and drug concentration data. CONCLUSION: Our findings suggest that CYP2D6 and ABCB1 G2677T and C3435T may be useful determinants of risperidone plasma concentrations, but the clinical implications of these associations in relation to treatment response and side-effects remain unclear.

**Bendelja K, Vojvoda V, Aberle N, Cepin-Bogović J, Gagro A, Mlinarić-Galinović G, et al. Decreased Toll-like receptor 8 expression and lower TNF-alpha synthesis in infants with acute RSV infection. *Respir Res.* 2010 Oct 14;11:143.**

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BACKGROUND: Toll-like receptors (TLRs) are part of the innate immune system, able to recognize pathogen-associated molecular patterns and activate immune system upon pathogen challenge. Respiratory syncytial virus (RSV) is a

RNA virus particularly detrimental in infancy. It could cause severe lower respiratory tract disease and recurrent infections related to inadequate development of anti-viral immunity. The reason could be inadequate multiple TLRs engagement, including TLR8 in recognition of single-stranded viral RNA and diminished synthesis of inflammatory mediators due to a lower expression.

METHODS: Intracellular TLR8 expression in peripheral blood monocytes from RSV-infected infants was profiled and compared to healthy adults and age matched controls. Whether the observed difference in TLR8 expression is a transitory effect, infants in convalescent phase (4-6 weeks later) were retested. Specific TLR8-mediated TNF- $\alpha$  production in monocytes during an acute and convalescent phase was analyzed.

RESULTS: RSV-infected and healthy infants had lower percentage of TLR8-expressing monocytes than healthy adults whereas decreased of TLR8 protein levels were detected only for RSV-infected infant group. Lower protein levels of TLR8 in monocytes from RSV-infected infants, compared to healthy infants, negatively correlated with respiratory frequency and resulted in lower TNF- $\alpha$  synthesis upon a specific TLR8 stimulation. In the convalescent phase, levels of TLR8 increased, accompanied by increased TNF- $\alpha$  synthesis compared to acute infection.

CONCLUSIONS: Lower TLR8 expression observed in monocytes, during an acute RSV infection, might have a dampening impact on early anti-viral cytokine production necessary to control RSV replication, and subsequently initiate an adaptive Th1 type immune response leading to severe disease in infected infants.