CROATIAN INTERNATIONAL PUBLICATIONS

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Zgonjanin D, Veselinović I, Kubat M*, Furač I*, Antov M, Lončar E et al. Sequence polymorphism of the mitochondrial DNA control region in the population of Vojvodina Province, Serbia. Leg Med (Tokyo). 2010;12:104-7.

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In order to generate and establish the database for forensic identification purposes in Vojvodina Province (Serbia), the sequence of the hypervariable regions 1 (HV1) and 2 (HV2) of the mtDNA control region were determined in a population of 104 unrelated individuals from Vojvodina Province, using a fluorescent-based capillary electrophoresis sequencing method. A total of 93 different haplotypes were found, of these 83 mtDNA types were unique, nine haplotypes were shared by two individuals and one haplotype by three individuals. The variation of mtDNA HV1 and HV2 regions was confined to 116 nucleotide positions, of which 72 were observed in the HV1 and 44 in the HV2. A statistical estimate of the results for this population showed the genetic diversity of 0.9977 and the random match probability of 1.18%. Haplogroup H was the most common haplogroup (43.3%). Haplogroups observed at intermediate levels included clusters U (13.5%), T (10.6%), J (8.6%) and W (5.8%).

Kovačić N, Grčević D, Katavić V, Lukić IK, Grubišić V, Mihovilović K et al. Fas receptor is required for estrogen deficiency-induced bone loss in mice. Lab Invest. 2010;90:402-13

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Bone mass is determined by bone cell differentiation, activity, and death, which mainly occur through apoptosis. Apoptosis can be triggered by death receptor Fas (CD95), expressed on osteoblasts and osteoclasts and may be regulated by estrogen. We have previously shown that signaling through Fas inhibits osteoblast differentiation. In this study we analyzed Fas as a possible mediator of bone loss induced by estrogen withdrawal. At 4 weeks after ovariectomy (OVX), Fas gene expression was greater in osteoblasts and lower in osteoclasts in ovariectomized C57BL/6J (wild type (wt)) mice compared with sham-operated animals. OVX was unable to induce bone loss in mice with a gene knockout for Fas (Fas -/- mice). The number of osteoclasts increased in wt mice after OVX, whereas it remained unchanged in Fas -/- mice. OVX induced greater stimulation of osteoblastogenesis in Fas -/- than in wt mice, with higher expression of osteoblast-specific genes. Direct effects on bone cell differentiation and apoptosis in vivo were confirmed in vitro, in which addition of estradiol decreased Fas expression and partially abrogated the apoptotic and differentiation-inhibitory effect of Fas in osteoblast lineage cells, while having no effect on Fas-induced apoptosis in osteoclast lineage cells. In conclusion, the Fas receptor has an important role in the pathogenesis of postmenopausal osteoporosis by mediating apoptosis and inhibiting differentiation of osteoblast lineage cells. Modulation of Fas effects on bone cells may be used as a therapeutic target in the treatment of osteoresorptive disorders.

Carev M, Valić M, Pecotić R, Karanović N, Valić Z, Pavlinac I, Đogaš Z. Propofol abolished the phrenic long-term facilitation in rats. Respir Physiol Neurobiol. 2010;170:83-90.

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The aim was to investigate the effect of propofol anesthesia on the phrenic long-term facilitation (pLTF) in rats. We hypothesized that pLTF would be abol-

ished during propofol-compared with urethane anesthesia. Fourteen adult, male, anesthetized, vagotomized, paralyzed, and mechanically ventilated Sprague-Dawley rats (seven per group), were exposed to the acute intermittent hypoxia (AIH) protocol. Peak phrenic nerve activity (PNA), burst frequency (f), and breathing rhythm parameters (Ti, Te, Ttot) were analyzed during the first hypoxia (TH1), as well as at 15 (T15), 30 (T30), and 60min (T60) after the final hypoxic episode, and compared to the baseline values. In propofol-anesthetized rats no significant changes of PNA were recorded after the last hypoxic episode, i.e. no pLTF was induced. There was a significant increase of PNA (59.4+/-6.6%, P<0.001) in urethane-anesthetized group at T60. AIH did not elicit significant changes in f, Ti, Te, Ttot in either group at T15, T30, and T60. The pLTF, elicited by AIH, was induced in the urethane-anesthetized rats. On the contrary, pLTF was abolished in the propofol-anesthetized rats.

Suštić A, Protić A, Cicvarić T, Župan Z. The addition of a brief ultrasound examination to clinical assessment increases the ability to confirm placement of double-lumen endotracheal tubes. J Clin Anesth. 2010 Jun;22(4):246-9.

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STUDY OBJECTIVE: To evaluate the role of a brief ultrasound examination (US) in detecting the correct position of the left double-lumen endotracheal tube (LDLT). DESIGN: Prospective, randomized clinical study. SETTING: Operating room of a university hospital. PATIENTS: 50 elective adult thoracic surgery patients who required a LDLT during anesthesia. INTERVENTION AND MEASUREMENTS: Patients were randomized to two groups: Group A, who underwent clinical assessment of the LDLT position, and Group B, who were examined clinically and by ultrasound. All 50 patients underwent the same conventional procedure of LDLT placement. In all patients, clinical assessment of LDLT positioning was made by observing chest wall expansion and checking lung compliance by manual ventilation and by auscultation of both lungs. In Group B, a very brief ultrasound (15-30 sec) examination was added. Ultrasound examination included visualization of the pleural movements ("lung sliding") and motion of the diaphragm from both sides before and after selective clamping of the bronchial and tracheal limbs. In both groups, a second anesthesiologist performed bronchoscopy to estimate actual LDLT position. MAIN RESULTS: Sensitivity and negative predictive values in detecting proper LDLT positioning for both methods were 100%. For the clinical assessment alone (Group A), specificity was 22%, accuracy was 72%, and positive predictive value, 70%; for the clinical and ultrasound assessment (Group B), specificity was 50%, accuracy was 88%, and positive predictive value, 86%. CONCLUSION: A brief ultrasound examination added to clinical assessment ensured more precise placement of LDLT than did clinical assessment alone.

Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. 2010 ;11:45-51.

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Autophagy is the cellular homeostatic pathway that delivers large cytosolic materials for degradation in the lysosome. Recent evidence indicates that autophagy mediates selective removal of protein aggregates, organelles and microbes in cells. Yet, the specificity in targeting a particular substrate to the autophagy pathway remains poorly understood. Here, we show that the mitochondrial protein Nix is a selective autophagy receptor by binding to LC3/GABARAP proteins, ubiquitin-like modifiers that are required for the growth of autophagosomal membranes. In cultured cells, Nix recruits GABARAP-L1 to damaged mitochondria through its amino-terminal LC3-interacting region. Furthermore, ablation of the Nix:LC3/GABARAP interaction retards mitochondrial clearance in maturing murine reticulocytes. Thus, Nix functions as an autophagy receptor, which mediates mitochondrial clearance after mitochondrial damage and during erythrocyte differentiation.

Boultwood J, Perry J, Zaman R, Fernandez-Santamaria C, Littlewood T, Kušec R*, et al. High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. Leukemia 2010;24:1139-45

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We have undertaken a genome-wide single nucleotide polymorphism (SNP) array analysis of 41 chronic myeloid leukemia (CML) patients. In total, 44 regions of uniparental disomy (UPD) >3 Mb were identified in 24 of 32 patients in chronic phase (CP), and 21 regions of UPD >3 Mb were identified in 13 of 21 patients in blast crisis (BC). Chromosome 8 had the highest frequency of UPD regions in both CP and BC samples. Eight recurrent regions of UPD

were observed among the 41 patients, with chromosome 8 showing the highest frequency. Ten regions of copy number change (CNC) >3 Mb were observed in 4 of 21 patients in BC, whereas none were observed in CP. We have identified several recurrent regions of UPD and CNC in CML that may be of pathogenetic importance. Overrepresentation of genomic aberrations (UPD and copy number gain) mapping to chromosome 8 was observed. Selected candidate genes mapping within the aberrant genomic regions were sequenced and mutation of the TP53 gene was observed in one case in BC and of the ASXL1 gene in 6 of 41 cases in CP or BC. Mutation of ASXL1 represents an important new molecular abnormality in CML.Leukemia advance online publication, 22 April 2010; doi:10.1038/leu.2010.65.

Galić E, Krpan D, Mirat J, Kušec V. Diversity of bone cell activity as a histomorphometric feature of idiopathic osteoporosis in men. Aging Male. 2010;13:18-24.

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Although osteoporosis in men is an increasing health problem, studies on osteoporosis in males are still scarce. The aim of our study was to determine the characteristics of bone tissue and bone turnover in men with idiopathic osteoporosis. Transiliac crest bone samples were histomorphometrically analyzed after double tetracycline labeling in 32 men aged 37-65 years who were diagnosed with idiopathic osteoporosis by densitometry of the lumbar spine and hip. Bone volume, osteoid surface, osteoblast surface, eroded surface, osteoid thickness, trabecular thickness, trabecular number, trabecular separation, and mineral apposition rate (MAR) were determined in all trabecular bone specimens. Bone volume and structural parameters indicated trabecular bone loss in most patients. Cellular parameters and MAR indicated variations in bone cell actions. No age-related decrease in histomorphometric parameters was found. After the patients were grouped according to MAR values, osteoblast and eroded surfaces were found to be lower in the group with decreased MAR values and elevated in the group of patients with increased MAR parameter. Trabecular thickness was greater in patients with lower than normal MAR, due to reduced resorption and probably loss of very thin trabeculae. Our results suggest that idiopathic osteoporosis in man resembles many characteristics of postmenopausal osteoporosis in women resulting in impaired trabecular structure due to unbalanced cellular activity and bone turnover rate.

Berković MC, Jokić M, Marout J, Radošević S, Zjacić-Rotkvić V, Kapitanović S. IL-2 -330 T/G SNP and serum values-potential new tumor markers in neuroendocrine tumors of the gastrointestinal tract and pancreas (GEP-NETs). J Mol Med. 2010;88:423-9.

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Cytokines participate in tumorigenesis of gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Single nucleotide polymorphisms (SNPs) in cytokine genes influence expression of proteins and are evaluated in cancer susceptibility. The aim of this study was to evaluate IL-2 -330 T/G SNP and susceptibility to GEP-NETs, and analyze the correlation between G-allele and IL-2 serum values in GEP-NET patients. Moreover we assessed the value of IL-2 as a tumor serum marker. IL-2 -330 T/G SNP was examined in 101 patients and 150 healthy volunteers and IL-2 serum levels in patients and 20 controls. Patients' IL-2 serum levels were compared to IL-2 -330 T/G genotypes and tumor functional status and finally with known markers such as chromogranin A (CgA) and 5-hydroxyindolacetic acid (5-HIAA). There was a significant difference in genotype distribution of the IL-2 -330 polymorphisms between GEP-NET and control group ($p = 0.0006$) as well as in the frequency of G-allele ($p = 0.010$). G-allele correlated with higher IL-2 serum levels ($p = 0.028$) and elevated in all patients, being highest in patients with functional tumors ($p = 0.039$). Compared to CgA and 5-HIAA, IL-2 was more specific in detecting GEP-NET patients ($p < 0.0001$ and $p < 0.0001$, respectively). Our results indicate importance of IL-2 in GEP-NET development and biochemical diagnosis.

Blagaić V, Houra K, Turčić P, Štambuk N, Konjevoda P, Boban-Blagaić A et al. The influence of alpha-, beta- and gamma-melanocyte stimulating hormone on acetaminophen induced liver lesions in male CBA mice. Molecules. 2010;15:1232-41.

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Research over the past decade has indicated that melanocortin peptides are potent inhibitors of inflammation and a promising source of new anti-inflammatory and cytoprotective therapies. The purpose of the present paper is to compare protective effects of alpha-, beta-, and gamma-melanocyte stimulating hormone on acetaminophen induced liver lesions in male CBA mice. Acetaminophen was applied intragastrically in a dose of 150 mg/kg, and tested

substances were applied intraperitoneally 1 hour before acetaminophen. Mice were sacrificed after 24 hours and intensity of liver injury was estimated by measurement of plasma transaminase activity (AST and ALT) and histopathological grading of lesions. It was found that alpha-, beta-, and gamma-MSH decrease intensity of lesions by both criteria in a dose-dependent manner.

Ćavar I, Kelava T, Vukojević K, Saraga-Babić M, Čulo F. The role of prostaglandin E2 in acute acetaminophen hepatotoxicity in mice. Histol Histopathol. 2010;25:819-30.

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Prostaglandin E2 (PGE_2), which is synthesized by many cell types, has a cytoprotective effect in the gastrointestinal tract and in several other tissues and cells. On the other hand, overdose or chronic use of a high dose of acetaminophen (Paracetamol, APAP) is a major cause of acute liver failure in the western world. These observations prompted us to investigate whether PGE_2 plays a role in host defence to toxic effect of APAP. (CBAT6T6xC57Bl/6)F1 hybrid mice of both sexes were intoxicated with a single lethal or high sublethal dose of APAP, which was administered to animals by oral gavage. Stabile analogue of PGE_2, 16,16-dimethyl PGE_2 ($dmPGE_2$), or inhibitor of its production, CAY10526, were given intraperitoneally (i.p.) 30 minutes before or 2 hours after APAP administration. The toxicity of APAP was determined by observing the survival of mice during 48 hours, by measuring concentration of alanine-aminotransferase (ALT) in plasma 20-22 hours after APAP administration and by liver histology. The results have shown that PGE_2 exhibits a strong hepatoprotective effect when it is given to mice either before or after APAP, while CAY10526 demonstrated mainly the opposite effect. Immunohistochemical or immunofluorescent examinations in the liver tissue generally support these findings, suggesting that PGE2 inhibited APAP-induced activation of nuclear factor kappa B (NF-kappaB). Similarly, PGE_2 down regulated the activity of inducible nitric oxide synthase (iNOS), which was up regulated by APAP. Thus, by these and perhaps by other mechanisms, PGE_2 contributes to the defence of the organism to noxious effects of xenobiotics on the liver.