CROATIAN INTERNATIONAL PUBLICATIONS

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Pavić M, Zadro R, Coen Herak D, Radić Antolić M, Dodig S: Gene frequencies of platelet-specific antigens in Croatian population. Transfus Med. 2010;20:73-7

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The human platelet antigens (HPA) are genetically defined polymorphisms expressed on platelet membrane glycoproteins. As platelet antigens are very important in several clinical situations and in population genetics, we used the polymerase chain reaction with sequence-specific primers (PCR-SSP) to investigate HPA-1, -2, -3 and -5 allele frequencies in the Croatian population. The HPA frequencies obtained in 219 Croatians were: 1a-0.854, 1b-0.146, 2a-0.890, 2b-0.110, 3a-0.575, 3b-0.425, 5a-0.895 and 5b-0.105. These data are similar to the frequencies reported in most European studies with some significant differences in HPA-2 when compared with the Dutch and German population, in HPA-3 when compared with the Swiss population and in HPA-5 when compared with the Finnish population. The three most common condensed HPA genotypes in the Croatian population were: HPA-1a/a, -2a/a, -3a/b, -5-a/a (0.283), HPA-1a/a, -2a/a, -3a/a, -5-a/a (0.137) and HPA-1a/b, -2a/a, -3a/b, -5-a/a (0.087). Data obtained in this study can be used for better understanding and treatment of immune-mediated platelet disorders in our population.

Mohr CA, Arapović J*, Mühlbach H, Panzer M, Weyn A, Dölken L et al. A spread-deficient cytomegalovirus for assessment of first-target cells in vaccination. J Virol. 2010;84:7730-42.

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Human cytomegalovirus (HCMV) is a human pathogen that causes severe disease primarily in the immunocompromised or immunologically immature individual. To date, no vaccine is available. We describe use of a spread-deficient murine CMV (MCMV) as a novel approach for betaherpesvirus vaccination. To generate a spread-deficient MCMV, the conserved, essential gene M94 was deleted. Immunization with MCMV-DeltaM94 is apathogenic and protective against wild-type challenge even in highly susceptible IFNalphabetaR(-/-) mice. MCMV-DeltaM94 was able to induce a robust CD4(+) and CD8(+) T-cell response as well as a neutralizing antibody response comparable to that induced by wild-type infection. Endothelial cells were identified as activators of CD8(+) T cells in vivo. Thus, the vaccination with a spread-deficient betaherpesvirus is a safe and protective strategy and allows the linkage between cell tropism and immunogenicity. Furthermore, genomes of MCMV-DeltaM94 were present in lungs 12 months after infection, revealing first-target cells as sites of genome maintenance.

Vitart V, Benčić G*, Hayward C, Škunca Herman K*, Huffman J, Campbell S et al. New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8. Hum Mol Genet. 2010;19:4304-11

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Central corneal thickness (CCT) is a highly heritable trait, which has been proposed to influence disorders of the anterior segment of the eye. A genome-wide association study (GWAS) of CCT was performed in 2269 individuals from three Croatian and one Scottish population. In the discovery set (1445 individuals), two genome-wide significant associations were identified for single nucleotide polymorphisms rs12447690 ($\beta = 0.23$ SD, $P = 4.4 \times 10^{-9}$) and rs1536482 ($\beta = 0.22$ SD, $P = 7.1 \times 10^{-8}$) for which the closest candidate genes (although ≥90 kb away) were zinc finger 469 (ZNF469) on 16q24.2 and collagen 5 alpha 1 (COL5A1) on 9q34.2, respectively. Only the ZNF469 association was confirmed in our replication set (824 individuals, $P = 8.0 \times 10^{-4}$) but COL5A1 remained a suggestive association in the combined sample ($\beta = 0.16$ SD, $P = 1.1 \times 10^{-6}$). Following a larger meta-analysis including recently published CCT GWAS summary data, COL5A1 was genome-wide significant ($\beta = 0.13$ SD, $P = 5.1 \times 10^{-8}$), together with two additional novel loci. The second new locus (defined by rs1034200) was 5 kb from the AVGR8 gene, encoding a putative transcription factor with typical ZNF and KRAB domains, in chromosomal region 13q12.11 ($\beta = 0.14$ SD, $P = 3.5 \times 10^{-9}$). The third new locus (rs6496932), on 15q25.3 ($\beta = 0.13$, $P = 1.4 \times 10^{-8}$), was within a wide linkage disequilibrium block extending into the 5′ end of the AKAP13 gene, encoding a scaffold protein concerned with

signal transduction from the cell surface. These associations offer mechanistic insights into the regulation of CCT and offer new candidate genes for susceptibility to common disorders in which CCT has been implicated, including primary open-angle glaucoma and keratoconus.

Ivković A, Pascher A, Hudetz D, Matičić D, Jelić M, Dickinson S et al. Articular cartilage repair by genetically modified bone marrow aspirate in sheep. Gene Ther. 2010;17:779-89

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Bone marrow presents an attractive option for the treatment of articular cartilage defects as it is readily accessible, it contains mesenchymal progenitor cells that can undergo chondrogenic differentiation and, once coagulated, it provides a natural scaffold that contains the cells within the defect. This study was performed to test whether an abbreviated ex vivo protocol using vector-laden, coagulated bone marrow aspirates for gene delivery to cartilage defects may be feasible for clinical application. Ovine autologous bone marrow was transduced with adenoviral vectors containing cDNA for green fluorescent protein or transforming growth factor (TGF)-beta1. The marrow was allowed to clot forming a gene plug and implanted into partial-thickness defects created on the medial condyle. At 6 months, the quality of articular cartilage repair was evaluated using histological, biochemical and biomechanical parameters. Assessment of repair showed that the groups treated with constructs transplantation contained more cartilage-like tissue than untreated controls. Improved cartilage repair was observed in groups treated with unmodified bone marrow plugs and Ad.TGF-beta1-transduced plugs, but the repaired tissue from TGF-treated defects showed significantly higher amounts of collagen II ($P<0.001$). The results confirmed that the proposed method is fairly straightforward technique for application in clinical settings. Genetically modified bone marrow clots are sufficient to facilitate articular cartilage repair of partial-thickness defects in vivo. Further studies should focus on selection of transgene combinations that promote more natural healing.

Hrgović I, Hrgović Z, Habek D*, Orešković S*, Hofmann J, Münstedt K. Use of complementary and alternative medicine in departments of obstetrics in Croatia and a comparison to Germany. Forsch Komplementmed. 2010;17:144-6

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BACKGROUND: This nationwide study assessed the use of complementary and alternative medicine (CAM) methods in departments of obstetrics in Croatia and compared it with an identical assessment carried out in Germany. METHODS: All Croatian obstetrics departments were sent a questionnaire already tested in Germany which assessed the use of CAM methods: whether any were used during childbirth and if so how frequently, and the reasons behind their application. RESULTS: Questionnaires were returned by 100% (36/36) of departments identified. The only used CAM therapy was acupuncture, which was available in 5.6% (2/36) of the departments. All other methods (homeopathy, aromatherapy, massage etc.) were not used at all. Furthermore, acupuncture was only administered by physicians. These findings were found to strongly contrast with the findings from Germany. CONCLUSIONS: Among the main CAM methods only acupuncture is used to a small extent in the field of obstetrics in Croatia. Thus, the impression from the literature that shows a considerable use of CAM in this area definitely does not apply for all parts of the world. Future studies should seek to identify the reasons behind the intensive use of CAM in Germany and its virtual non-use in Croatia. This also means that analyses of CAM use are required with respect to perinatal outcome, the results of which could finally help decide about the reasonability of CAM.

Nedić G, Nikolac M, Šviglin KN, Muck-Šeler D, Borovečki F, Pivac N. Association study of a functional catechol- O-methyltransferase (COMT) Val108/158Met polymorphism and suicide attempts in patients with alcohol dependence. Int J Neuropsychopharmacol. 2010;22:1-12

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Alcohol dependence is frequently associated with aggressive and suicidal behaviour. Genetic factors contribute to both behaviours. Candidate genes, related to suicide and aggression, include genes involved in serotonin, norepinephrine and dopamine pathways. The enzyme catechol-O-methyl transferase (COMT) degrades dopamine, epinephrine and norepinephrine. The functional polymorphism (COMT Val108/158Met) affects COMT activity, with the valine (Val) variant associated with higher and the methionine (Met) variant with lower COMT activity. This polymorphism is associated with aggressive and suicidal behaviour, but the literature data on this relationship is contradictory and

inconsistent. The hypothesis of this study was that Met allele carriers with alcohol dependence will have a higher frequency of suicide attempts compared to other genotypes. Participants were 312 male and 81 female medication-free patients with alcohol dependence and 487 male and 122 female unrelated, non-suicidal medication-free Caucasian healthy subjects. Our results showed significant (χ^2 test with standardized residuals) differences in the frequencies of COMT variants in all alcoholics, alcoholics with different comorbid diagnoses, and in male but not in female alcoholics, with or without suicide attempts. Male alcoholic suicide attempters, compared to male non-attempters, had the higher frequency of Met/Met genotype or Met allele, and significantly (Kruskal-Wallis ANOVA on ranks and Mann-Whitney test) higher aggression and depression scores. These results confirmed the associations between Met allele and aggressive behaviour or violent suicide attempts in various psychiatric diagnoses, and suggested that Met allele of the COMT Val108/158 Met might be used as an independent biomarker of suicidal behaviour across different psychopathologies.

Budišić M, Karlović D, Trkanjec Z, Lovrenčić-Huzjan A, Vuković V, Bošnjak J et al. Brainstem raphe lesion in patients with major depressive disorder and in patients with suicidal ideation recorded on transcranial sonography. Eur Arch Psychiatry Clin Neurosci. 2010;260:203-8

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Recent transcranial sonography (TCS) studies showed that disruption of echogenic midbrain line, corresponding to basal limbic system and raphe nuclei (RN) within, might represent functional marker for the development of depression. Major depressive disorder (MDD) is one of the most common psychiatric disorders associated with suicidal ideation. We initiated this study to assess the usefulness of TCS recording in a group of MDD patients and in MDD patients who also reported suicidal ideation, on the assumption that TCS might serve as a screening method for differentiating patients at risk of suicide. Altogether 71 subjects: 17 patients with MDD, 14 patients with MDD who also reported suicidal ideation and 40 healthy controls, were studied using TCS by two independent physicians. Reduced raphe echogenicity was found in 8 of 17 (47%) of the patients with MDD but only in 6 of 40 (15%) controls. In patients with suicidal ideations that finding was even more pronounced (12 of 14, 86%) with the highest frequency of completely not visible TCS RN finding (10 of 14, 72%). Data showed that altered echogenicity of the RN is frequent in patients with suicidal ideation. Normal RN echogenicity in MDD patients was associated with less severe depressive symptoms and rarely with the presence of suicidal ideations. As far as we know, these are the first ever obtained results which show that TCS might help differentiating MDD patients with suicidal risk or eventually predict good disease recovery based on the findings of RN hypo- or normoechogenicity.

Bendelja K, Vojvoda V, Aberle N, Čepin-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;11:143:

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.