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Knežević A, Polašek O, Gornik O, Rudan I, Campbell H, Hayward C et al. Variability, heritability and environmental determinants of human plasma N-glycome. VJ Proteome Res. 2009;8:694-701.

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Plasma glycans were analyzed in 1008 individuals to evaluate variability and heritability, as well as the main environmental determinants that affect glycan structures. By combining HPLC analysis of fluorescently labeled glycans with sialidase digestion, glycans were separated into 33 chromatographic peaks and quantified. A high level of variability was observed with the median ratio of minimal to maximal values of 6.17 and significant age- and gender-specific differences. Heritability estimates for individual glycans varied widely, ranging from very low to very high. Glycome-wide environmental determinants were also detected with statistically significant effects of different variables including diet, smoking and cholesterol levels.

Heusser K, Džamonja G*, Tank J, Palada I*, Valić Z*, Baković D* et al. Cardiovascular regulation during apnea in elite divers. Hypertension. 2009;53:719-24.

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Involuntary apnea during sleep elicits sustained arterial hypertension through sympathetic activation; however, little is known about voluntary apnea, particularly in elite athletes. Their physiological adjustments are largely unknown. We measured blood pressure, heart rate, hemoglobin oxygen saturation, muscle sympathetic nerve activity, and vascular resistance before and during maximal end-inspiratory breath holds in 20 elite divers and in 15 matched control subjects. At baseline, arterial pressure and heart rate were similar in both groups. Maximal apnea time was longer in divers (1.7±0.4 versus 3.9±1.1 minutes; $P<0.0001$), and it was accompanied by marked oxygen desaturation (97.6±0.7% versus 77.6±13.9%; $P<0.0001$). At the end of apnea, divers showed a >5-fold greater muscle sympathetic nerve activity increase ($P<0.01$) with a massively increased pressor response compared with control subjects (9±5 versus 32±15 mm Hg; $P<0.001$). Vascular resistance increased in both groups, but more so in divers (79±46% versus 140±82%; $P<0.01$). Heart rate did not change in either group. The rise in muscle sympathetic nerve activity correlated with oxygen desaturation ($r^2=0.26$; $P<0.01$) and with the increase in mean arterial pressure ($r^2=0.40$; $P<0.0001$). In elite divers, breath holds for several minutes result in an excessive chemoreflex activation of sympathetic vasoconstrictor activity. Extensive sympathetically mediated peripheral vasoconstriction may help to maintain adequate oxygen supply to vital organs under asphyxic conditions that untrained subjects are not able to tolerate voluntarily. Our results are relevant to conditions featuring periodic apnea.

Ivančev V, Baković D, Obad A, Brešković T, Palada I, Joyner MJ et al. Effects of indomethacin on cerebrovascular response to hypercapnea and hypocapnea in breath-hold diving and obstructive sleep apnea. Respir Physiol Neurobiol. 2009;166:152-8.

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We tested whether breath hold divers (BHD) and obstructive sleep apnea (OSA) subjects had similar middle cerebral artery velocity (MCAV) responses to hypercapnea and hypocapnea. We analyzed changes in MCAV (cm/s) in response to hypocapnea and hyperoxic hypercapnea during placebo or after 90min of oral indomethacin (100mg) in BHD (N=7) and OSA (N=7). During control hypercapnea MCAV increased for 54.4% in BHD and 48.4% in OSA. Indomethacin blunted the MCAV increase in

response to hypercapnea in BHD (*P*=0.02), but not in OSA. Indomethacin attenuated the mean arterial pressure response in BHD, but not in OSA. The blunted MCAV responses to hypercapnea with indomethacin in BHD, but not in OSA patients suggests that (a) the normal contribution of local vasodilating mechanisms to the cerebrovascular responses to hypercapnea is absent in OSA patients and (b) exposure to chronic/repeated apneas is not causal per se in limiting the contribution of vasodilating mechanisms to the cerebrovascular responses to hypercapnea in OSA.

Majhen D, Nemet J, Richardson J, Gabrilovac J, Hajsig M, Osmak M, Eloit M, Ambriović-Ristov. A Differential role of alpha(v)beta(3) and alpha(v)beta(5) integrins in internalization and transduction efficacies of wild type and RGD4C fiber-modified adenoviruses. Virus Res. 2009;139:64-73.

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In order to analyze the role of alpha(v)beta(3) and alpha(v)beta(5) integrins in gene transfer by adenovirus-based vectors, an RGD4C motif was inserted into the HI-loop of wild type or shortened fiber protein of human adenovirus of serotype 5, thereby creating Ad5RGD4C and Ad5Delta639RGD4C vectors, respectively. Infection by the latter is independent of the Coxsackie B adenovirus receptor. Internalization and transduction of these vectors and were investigated in several stably transfected cell clones derived from a human laryngeal carcinoma cell line (HEp2) expressing different ratios of alpha(v)beta(5) and alpha(v)beta(3) integrins. We show that alpha(v)beta(3) is more successful than alpha(v)beta(5) in: (i) mediating adenovirus internalization and transduction when the RGD motif is present only in the penton base and (ii) mediating internalization and transduction by RGD4C-fiber modified adenoviruses. The highest amount of internalized virus was found in the cell clone in which alpha(v)beta(3) integrin predominated over alpha(v)beta(5) integrin (as judged by the % of cells expressing alpha(v)beta(3) and alpha(v)beta(5) integrins). However the level of transgene expression in this cell line was even lower than that in parental HEp2 cells which do not express alpha(v)beta(3) integrin. This discrepancy between internalization and transgene expression (transduction) is likely due to the crucial role of alpha(v)beta(5) in membrane permeabilization, indicating that alpha(v)beta(5) integrin is a limiting factor for Ad5-mediated gene transfer. We conclude that alpha(v)beta(3) integrin is an efficient adenovirus internalization receptor, but cannot functionally replace alpha(v)beta(5) in endosomal release.

Zahradka K, Buljubasić M, Petranović M, Zahradka D. Roles of ExoI and SbcCD nucleases in "reckless" DNA degradation in recA mutants of Escherichia coli. J Bacteriol. 2009;191:1677-87.

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Exponentially growing recA mutant cells of Escherichia coli display pronounced DNA degradation that starts at the sites of DNA damage and depends on RecBCD nuclease (ExoV) activity. As a consequence of this "reckless" DNA degradation, populations of recA mutants contain a large proportion of anucleate cells. We have found that both DNA degradation and anucleate-cell production are efficiently suppressed by mutations in the xonA (sbcB) and sbcD genes. The suppressive effects of these mutations were observed in normally grown, as well as in UV-irradiated, recA cells. The products of the xonA and sbcD genes are known to code for the ExoI and SbcCD nucleases, respectively. Since both xonA and sbcD mutations are required for strong suppression of DNA degradation while individual mutations have only a weak suppressive effect, we infer that ExoI and SbcCD play partially redundant roles in regulating DNA degradation in recA cells. We suggest that their roles might be in processing (blunting) DNA ends, thereby producing suitable substrates for RecBCD binding.

Vugrek O, Beluzić R, Nakić N, Mudd SH. S-adenosylhomocysteine hydrolase (AHCY) deficiency: two novel mutations with lethal outcome. Hum Mutat. 2009;30:E555-65.

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This paper reports studies of two novel, allelic missense mutations found in the S-adenosylhomocysteine hydrolase (AHCY) gene from a new case of AHCY deficiency in an infant girl who died at age four months. The mutations lead to replacement of arginine with cysteine (p.Arg49Cys) and aspartic acid with glycine (p.Asp86Gly). Functional analysis of recombinant proteins containing the mutations detected showed that both dramatically reduce AHCY activity. The p.Arg49Cys mutant protein forms intermolecular disulphide bonds, leading to macromolecular structures that can be prevented by reducing agent DTT. The p.Asp86Gly protein tends to form enzymatically inactive aggregates

and the loss of a single negative charge as a result of the mutation is involved in enzyme inactivation. We show that replacing Gly86 with negatively charged Glu86 in mutant protein restores enzymatic activity to 70% of wild-type, whereas changing Gly86 to positively charged Lys86 or uncharged Leu86 does not improve enzyme activity, indicating that the negative charge is important for maintenance of such activity. These studies significantly extend knowledge about the importance of residue 86 for AHCY activity. Residue 86 has not been implicated before in this way and the results suggest that the present model of S-adenosylhomocysteine (AdoHcy) hydrolysis may need refinement. Our functional studies provide novel insight into the molecular defect underlying AHCY deficiency and reveal that both low enzyme activity and protein stability of AHCY contribute to the clinical phenotype.

Petrović V, Pérez-García C, Pasantes JJ, Satović E, Prats E, Plohl M. A GC-rich satellite DNA and karyology of the bivalve mollusk Donax trunculus: a dominance of GC-rich heterochromatin. Cytogenet Genome Res. 2009;124:63-71.

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We characterized the DTF2 satellite DNA family of the clam Donaxtrunculus and compared its chromosomal localization with cytogenetic data revealed by fluorochrome banding, C-banding, and 28S rDNA FISH. In contrast to the other satellites detected previously in this species, DTF2 is an abundant (2%) GC-rich satellite that exhibits CpG methylation. Sequence characteristics of DTF2 indicate that its evolution is not affected by constraints that might indicate some functional interactions. Fluorescence in situ hybridization revealed subtelomeric location of this satellite on a subset of 14 out of 19 D. trunculus chromosome pairs. The chromomycin A(3) (CMA) staining of GC-rich regions on D. trunculus chromosomes revealed a complex banding pattern that overlaps completely with C-bands. In total, only three bands show subtelomeric location, while 13 bands are located interstitially, one of them being coincident with the 28S rDNA hybridization signal. No bands, either CMA positive (GC-rich) or DAPI positive (AT-rich) were detected at centromeric chromosomal positions. Only two of the CMA-positive bands co-localize with the DTF2 satellite, showing a) the presence of small islands of GC-rich repetitive sequences that remained undetected by CMA/C-banding and b) the abundance of DTF2-divergent GC-rich sequences at interstitial chromosomal locations. Copyright 2009 S

Barkić M, Crnomarković S, Grabušić K, Bogetić I, Panić L, Tamarut S et al. The p53 tumor suppressor causes congenital malformations in Rpl24-deficient mice and promotes their survival. Mol Cell Biol. 2009;29:2489-504.

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Hypomorphic mutation in one allele of ribosomal protein l24 gene (Rpl24) is responsible for the Belly Spot and Tail (Bst) mouse, which suffers from defects of the eye, skeleton, and coat pigmentation. It has been hypothesized that these pathological manifestations result exclusively from faulty protein synthesis. We demonstrate here that upregulation of the p53 tumor suppressor during the restricted period of embryonic development significantly contributes to the Bst phenotype. However, in the absence of p53 a large majority of Rpl24(Bst/+) embryos die. We showed that p53 promotes survival of these mice via p21-dependent mechanism. Our results imply that activation of a p53-dependent checkpoint mechanism in response to various ribosomal protein deficiencies might also play a role in the pathogenesis of congenital malformations in humans.

Peternel S, Pilipović K, Župan G. Seizure susceptibility and the brain regional sensitivity to oxidative stress in male and female rats in the lithium-pilocarpine model of temporal lobe epilepsy. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:456-62.

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Several studies have shown the existence of sex differences in the sensitivity to various convulsants in animals and to the development of some epilepsy types in humans. The purpose of this study was to investigate whether there are sex differences in seizure susceptibility and sensitivity of different brain regions to oxidative stress in rats with status epilepticus (SE) induced by lithium-pilocarpine administration, that provides a common experimental model of temporal lobe epilepsy (TLE) in humans. Latencies to isolated full limbic seizures or SE onset as well as the number of the animals presenting full limbic seizures, SE or full limbic seizures that progressed to SE were recorded for 2 h after pilocarpine administration. Number of animals which survived 24 h after SE onset was also monitored. Levels of lipid peroxidation as well as the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the piriform and entorhinal cortices, temporal neo-

cortex, thalamus, and hippocampus in rats of both sexes, at 24 h after SE onset were determined. Results of our study showed that males developed full limbic seizures and SE more rapidly and in greater number than females. Levels of lipid peroxidation in all brain regions examined, the SOD activities in the piriform and entorhinal cortices, and temporal neocortex as well as the GSH-Px activities in the piriform and entorhinal cortices, and thalamus were significantly higher in rats with SE in comparison to the values of mentioned biochemical parameters in rats of the control groups. Lipid peroxidation level in the temporal neocortex as well as the GSH-Px activity in the hippocampus in male rats were significantly higher in comparison to the values registered in females. With the exception of the thalamus, where SOD activity in male rats with SE was significantly higher in relation to the respective control group and also to females with SE, sex differences in the response of other brain regions investigated to oxidative stress were not obtained, at 24 h after SE.

Mlinarić-Galinović G, Vilibić-Čavlek T, Ljubin-Šternak S, Draženović V, Galinović I, Tomić V et al. Eleven consecutive years of respiratory syncytial virus outbreaks in Croatia. Pediatr Int. 2009;51:237-40.

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BACKGROUND: Respiratory syncytial virus (RSV) is the most common cause of severe lower respiratory tract infections (LRTI) in infants. The aim of the present study was to analyze the epidemiologic characteristics of RSV outbreaks in Croatian children. METHODS: Over a period of 11 consecutive years (1994-2005), 3435 inpatients with acute respiratory infections (ARI) aged from birth to 10 years and were residing in Zagreb County were tested for infection with RSV and other respiratory viruses at the Virology Department, Croatian National Institute of Public Health. RSV was identified in nasopharyngeal secretions by isolation on cell culture and/or detection with monoclonal antibodies using a direct fluorescence assay. RESULTS: RSV was the most common causative agent of ARI (42.2%; 658/1559) for the infants 0-6 months of age. It was also the etiologic agent of LRTI in 49% (495/1010) of infants of similar age. RSV was demonstrated in 56.5% (382/676) of infants with bronchiolitis, and in 36.5% (49/134) of those with pneumonia in this age group. CONCLUSION: The overall prevalence of RSV infection in Croatian children with acute respiratory illness, and its occurrence in various age groups, has remained stable over the past decade. RSV was found to be the most common cause of bronchiolitis occurring throughout childhood (52.7%; 482/913).