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A SHORT TANDEM REPEAT IN THE MACROPHAGE MIGRATION INHIBITORY FACTOR GENE PROMOTER IS ASSOCIATED WITH ACUTE LUNG INJURY SUSCEPTIBILITY IN A SPANISH POPULATION. C. Flores, S. Ma, K. Maresso, S. Devender, J. Theisen-Toupal, J. Villar, J.G. Garcia, University of Chicago, Chicago, IL; Canary Islands, Spain.

Rationale: Acute lung injury (ALI) is a complex inflammatory syndrome with presumed genetic predisposition. Macrophage migration inhibitory factor (MIF) potentially plays a major role in ALI pathogenesis and constitutes a candidate gene for ALI susceptibility. As the short tandem repeat (STR) 5-repeat allele at −794 of the *MIF* gene reported decreases *MIF* promoter activity, we examined this STR and the complete SNP variation of the *MIF* gene in association with susceptibility to ALI in a Spanish population. **Methods:** DNA samples from 96 healthy controls and 80 severe septic patients were used to genotype the *MIF* STR and to sequence the entire gene and the ≈2 kb flanking regions. *Polyphred* 6.0 software was used to detect SNPs, and variation was examined for linkage disequilibrium (LD) using *Haploview* 3.32. Odds ratio (OR) and 95% confidence interval (CI) for association of individual polymorphisms were assessed with SNPstats. Haplotype associations were evaluated by a sliding-window approach. **Results:** The 5-allele homozygous genotype at −794 STR was associated with an OR = 11.9 (95% CI 1.4–98.9, $p = .01$) after inclusion of covariates in the multiple logistic regression model. Sequencing revealed a total of 45 polymorphic loci (13 novel) that were also analyzed for association. None of them were significantly associated with ALI, either individually or in haplotypes. This result is congruent with the fact that neither SNPs nor haplotypes are in strong LD with the STR. **Conclusions:** Our data suggest that the *MIF* gene is associated with susceptibility to ALI and support the causality of the STR locus. Genotyping of this STR is required for future association studies as the *MIF* SNPs examined were not in strong LD with the STR variation.

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POSTPRANDIAL HEMODYNAMIC RESPONSES IN YOUNGER PATIENTS WITH NEUROGENIC ORTHOSTATIC HYPOTENSION. J.L. Gilden, A. Garbharran, S. Patel, P. Patil, J. Shah, V. Vartanian, N. Shukla, S.P. Singh, RFUMS/Chicago Medical School, North Chicago, IL.

Background: Previous studies have documented the presence of various neurohormonal changes in patients with symptomatic neurogenic orthostatic hypotension (NOH) (blood pressure decrease upon standing = 20 mm Hg systolic [SBP] and/or 10 mm Hg diastolic [DBP] with symptoms of dizziness, lightheadedness, or syncope). Although postprandial hypotension is exacerbated in the older patient with autonomic dysfunction (AN), it has not been well documented in younger patients with NOH. **Methods:** Orthostatic tolerance (SBP/DBP) was measured in response to a standard carbohydrate challenge using a 5-hour 75 g oral glucose tolerance test in 23 younger NOH patients (17 female, 6 male) (aged 43 ± 9 years) (diagnoses = postural orthostatic tachycardia syndrome/orthostatic intolerance, autoimmune, and mitral valve prolapse). AN was documented by abnormal RR inspiratory:expiratory ratios, Q–Tc, and 30:15 ratios. Results show that there were orthostatic decreases of SBP/DBP in response to a glucose challenge in the entire group that were greatest for SBP by 5 hours and DBP by 3 hours (−12 ± 2 mm Hg/−8 ± 2 mm Hg) ($p < .05$). However, the patterns of orthostatic decreases in females showed continual SBP/DBP decreases with the maximal decrease occurring by 5 hours for SBP and 3 hours for DBP, whereas the pattern of changes in males demonstrated earlier SBP decreases by 2 hours and 1½ hours for DBP, which remained by 5 hours. Symptoms of OH were also exacerbated in the postprandial state. **Conclusion:** Although postprandial hypotension is a well-known feature of NOH in older patients, younger NOH patients also demonstrate similar responses to a glucose challenge. The patterns of these orthostatic changes may be different depending upon gender.

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ASSOCIATION BETWEEN THE T-786C ENOS POLYMORPHISM AND IDIOPATHIC OSTEONECROSIS OF THE HIP. C.J. Glueck, R.A. Freiberg, J. Oghene, N. Fontaine, P. Wang, Jewish Hospital of Cincinnati, Cincinnati, OH.

Nitric oxide (NO) regulates bone turnover by osteoblasts-osteoclasts. NO production is impaired by T-786C eNOS and stromelysin 1 5A6A polymorphisms that lead to vasoconstriction, platelet aggregation, reduced angiogenesis, and bone formation, all of which may be associated with osteonecrosis (ON) of the hip. eNOS and stromelysin polymorphisms were compared in 95 hip ON cases (54 idiopathic [18 smokers], 41 secondary [9 smokers]) and 72 healthy normal adult controls. TT eNOS homozygosity was present in 11 of 54 (20%) idiopathic ON cases versus 1 of 51 (2%) race-/gender-/age-matched controls, TC heterozygosity in 28 of 54 (52%) cases versus 17 of 51 (33%) controls, and CC (wild-type normal) in 15 of 54 (28%) cases versus 33 of 51 (65%) controls, $p = .0001$. The eNOS mutant allele frequency in idiopathic ON was 50 of 108 (46%), higher than in race-/gender-/age-matched controls, 19 of 102 (19%), $p < .0001$. The case-control TT versus CC odds ratio (OR) was 24.2 ($p = .0002$), 95% CI 2.86–204.9, TC versus CC OR was 3.62 ($p = .003$), 95% CI 1.54–8.54, and TT versus combined TC with CC OR was 8.78 ($p = .003$), 95% CI 1.59–103.1. By logistic regression, the eNOS T-786 mutant allele was independently associated with idiopathic ON ($p < .0001$), OR 4.59, 95% CI 2.26–9.34. Secondary ON cases did not differ from 41 race-/gender-/age-matched controls in the distribution of the T-786C eNOS polymorphism ($p = .52$) or in mutant allele frequency (24% vs 23%, $p = .85$). The 54 cases with idiopathic ON differed from 41 cases with secondary ON, more likely to have mutant TT eNOS genotypes ($p = .008$) and having a higher mutant T allele frequency ($p = .002$). Idiopathic and secondary ON did not differ from controls for the distribution of the stromelysin 1 5A6A polymorphism ($p > .7$) or for the 6A allele frequency ($p > .4$). The eNOS T-786C polymorphism is associated with and may contribute to the pathogenesis of idiopathic ON.

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POSITIVE INFLUENCE OF SUPPLEMENTAL MANGANESE ON IRON INCORPORATION IN BRAIN AND RED BLOOD CELLS. S. Gupta, G.M. Stroh, H. Hassouna, Michigan State University, East Lansing, MI.

Structural, biochemical, and physiologic similarities make it possible for manganese to directly interact with iron on enzymes and proteins that require iron as a cofactor in their catalytic center. Elegant in vitro studies by Li et al (Toxicology and Applied Pharmacology 2005;205:188–200) demonstrate that manganese treatment alters the transcriptional but not translational level of the transferrin receptor (Tfr) expression and significantly augments the influx of Fe to the choroid plexus at the blood-CSF barrier. Transferrin bound diferric iron interacts with Tfr to undergo receptor-mediated endocytosis into erythroid precursors, hepatocytes, and brain endothelial cells, and proteolytic cleavage of the extracellular Tfr segment provides an index of the iron tissue levels. Although manganese toxicity is well documented, a beneficial role for manganese on iron homeostasis in the blood and the brain has never been reported. We present a lifelong iron deficiency microcytic anemia associated with symptoms suggestive of neurotransmitter dysregulation and perceptual size distortion in a 54-year-old white female. She had a total absence of sweating, severe constipation, and intolerance to heat and cold and episodes of oculomotor bias with size underestimation lasting 15 to 20 minutes. The anemia and symptoms resolved with daily administration of 10 mg over-the-counter oral manganese supplement (manganese). Premanganese levels for haptoglobin, direct bilirubin, and immunoglobulin were within the normal range. Erythrocyte sedimentation rate and platelet and white cell counts were within the normal range. Erythropoietin levels were 11 mU/mL (ref 4–21 mU/mL). Blood transfusions but not oral iron improved her symptoms but not the red cell indices and a consistently low reticulocyte count (0.6%). She had been off oral iron for a few months prior to her visit. Manganese was started in May 2005, discontinued December to February 2006 (‡), and resumed from then until the present. Manganese-induced changes in her hematologic profile and iron status from April 2005 to November 2006 are presented in Table 1. Studies performed in March and April in 2005 show iron studies consistent with appropriate iron absorption from the gut and inefficient incorporation of iron in the erythron, possibly from decreased TfR expression. Her tissue iron stores measured by ferritin levels were negligible. Manganese significantly increased her Hgb levels and corrected the MCV but not the soluble transferrin receptor levels that remain consistently above normal, an indication of deficient iron tissue storage. She started perspiring, and her ocular symptoms did not recur. On August 6, 2006, she had significant blood loss (§) that decreased her red blood cell count to 2.8 mill/μg and created a normochromic normocytic anemia with a reticulocyte count rising to 2%, a surprising consequence of manganese. We postulate that manganese, by positively influencing Tfr expression, reversed the underlying microcytic hypochromic iron deficiency anemia and related symptoms.

TABLE 1 Manganese-Induced Changes in Hematologic Profile and Iron Status

	Hgb g/dL 13.5–16.5	*MCV fl 80–100*	*Hematocrit % 36–45*	*RBC mill/μg 3.5–5.5*	*Retic % 0.2–2*	*Total Iron μg/dL 50-150*	*TIBC μg/dL 270–440*	*Iron Saturation % 20–50*	*Soluble Transferin Receptor mg/L 1.8–4.1*
Mar '05	7.4	68	26	3.77	0.6				
Apr '05	9.7	72	32	4.53	0.6	268	446	60	
May '05	11.0	84	37	4.15					4.1
Aug '05	13.6	87	39	4.46					5.7
Sep '05	14.4	94	42	4.45		81	385	21	
Feb '06‡	12.7	88	40	4.59	0.6	398	510	78	4.8
Mar '06	12.7	85	39	4.59		90	427	21	5.1
May '06	14.7	89	43	4.77	0.6				5.1
Aug '06§	9.7	94	27	2.88	2.0	21	299	7	3.0
Sep '06	11.1	89	34	3.81	0.8				13.4
Oct '06	10.5	86	32	3.86	0.8				11.4
Nov '06	13	85	40	4.7	0.3				10

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KRUPPEL-LIKE FACTOR 4 REGULATES ENDOTHELIAL INFLAMMATION. A. Hamik, Z. Lin, A. Kumar, M. Balcells, S. Sinha, J. Katz, R.E. Gerzsten, E.R. Edelman, M.K. Jain, Case Western Reserve University, Cleveland, OH; Boston, MA; Cambridge, MA; Charlestown, MA; Philadelphia, PA.

Background: The vascular endothelium plays a critical role in vascular homeostasis. A limited proinflammatory, prothrombotic state is appropriate in the context of infection or wound healing, but sustained endothelial activation leads to deleterious conditions such as atherosclerosis and pathologic thrombosis. Inflammatory cytokines and nonlaminar blood flow induce endothelial dysfunction and confer a proadhesive and prothrombotic phenotype. Therefore, identification of factors that mediate the effects of these stimuli on endothelial function is of considerable interest. Kruppel-like factors (KLFs) are a subclass of the zinc-finger family of transcription factors. Previous studies demonstrate that KLF proteins typically regulate critical aspects of cellular differentiation and tissue development. Studies from our laboratory and others demonstrate an emerging role for this family of transcriptional regulators in vascular biology. KLF4/GKLF (gut-enriched Kruppel-like factor) was first identified as being highly expressed in epithelial cells, and subsequent work has verified a critical role in skin and intestinal development. KLF4 has also been identified in endothelial cells, yet its function in vessel biology has yet to be elucidated. **Methods and Results:** Immunohistochemical analysis of mouse and human vascular tissues demonstrates the expression, in vivo, of KLF4 in human and mouse endothelial cells. Northern blot analysis of total mRNA harvested from primary endothelial cell lines derived from various human arterial vascular beds (aorta, pulmonary artery, and umbilical artery) shows expression of endothelial KLF4 in a variety of arterial and venous cell lines. Furthermore, we demonstrate that endothelial KLF4 is induced by proinflammatory stimuli and shear stress. Overexpression of KLF4 induces expression of multiple anti-inflammatory and antithrombotic factors, including eNOS and thrombomodulin, and inhibits basal and cytokine-mediated expression of a diverse set of proinflammatory factors, including MCP-1, RANTES, CRP, PAI-1, IL-6, and IL-8. The significance of endogenous expression of KLF4 on target genes was assessed in experiments using siRNA-mediated knockdown of KLF4. These experiments demonstrate that KLF4 depletion leads to enhancement of TNF-α-induced VCAM-1 and tissue factor expression. In addition to the determination of target genes, we have verified the functional importance of KLF4 by demonstrating that KLF4 expression markedly decreases inflammatory cell adhesion to the endothelial surface and prolongs clotting time under inflammatory states. As a first step toward understanding the molecular basis of KLF4-mediated regulation of endothelial target genes, we assessed the effect of KLF4 on target gene promoters. KLF4 differentially regulates the promoter activity of pro- and anti-inflammatory genes in a manner consistent with its anti-inflammatory function. **Conclusion:** Inflammatory cytokines and the biomechanical effects of laminar shear stress are the most potent effectors of endothelial homeostasis identified to date. Perturbation of endothelial function by proinflammatory cytokines or nonlaminar

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