1079-130 Evidence for Association of the Glu298Asp and T786C Polymorphisms of the Endothelial Nitric Oxide Synthase Gene to the Presence and Severity of Coronary Artery Disease

Maria Giovanna Colombo, Maria Grazia Andreassi, Nicoletta Botto, Samantha Manfredi, Serena Masetti, Umberto Paradossi, Aldo Clerico, Andrea Biagini, CNR, Institute of Clinical Physiology, G. Pasquinucci Hospital, Massa, Italy

Background: Genetic variants of the endothelial Nitric Oxide Synthase(eNOS) could influence individual susceptibility to coronary artery disease (CAD).
Objective: This study investigated the relationship of the eNOS Glu298Asp and T786C polymorphisms, both alone and in combination, to the presence and severity of CAD in the Italian population.
Methods: We analyzed the eNOS Glu298Asp and T786C variants in 415 and 374, respectively, unrelated subjects who underwent coronary angiography. The severity of CAD was expressed by means of the Duke scoring system.
Results: There was significant linkage disequilibrium between the two eNOS gene polymorphisms ($p<0.0001$). The Glu298Asp variant was significantly associated with the occurrence ($p=0.03$) and severity of CAD ($p=0.01$). The T786C polymorphism was significantly associated with the presence ($p=0.02$) and with the extent and severity of CAD (39.6±2.9, 38.1±2.2 and 27.3±2.8 for CC786, TC786 and TT786, respectively; $p=0.004$). The risk of CAD was increased among CC786 carriers in comparison to homozygous subjects for the T786 allele (OR 2.5; $p<0.01$) and was independent from the other common risk factors ($p=0.04$). Moreover, carriers of both Asp/Asp298 and at least one C786 allele were at higher risk of CAD than carriers of single genotypes (OR 4.0 for Asp/Asp and C786 allele simultaneously carriers vs 2.6 and 1.7 for Asp/Asp and for C786 allele, respectively).
Conclusions: The Glu298Asp and the T786C polymorphisms of the eNOS gene are associated with the presence, extent and severity of CAD in the Italian population.

1079-131 The A376G (A+) Variant of the Glucose-6-Phosphate Dehydrogenase Gene Is Associated With Endothelial Dysfunction in African Americans

Marc A. Forgione, Joseph Loscalzo, Monica Holbrook, Rowlens Melduni, Joseph Palmisano, Carolyn Maxwell, Clinton Baldwin, Joseph A. Vita, Boston University School of Medicine, Boston, MA

Glucose-6-phosphate dehydrogenase (G6PD) is the primary source of NADPH in cells. G6PD deficiency is an X-linked enzymopathy that occurs in ~11% of African Americans and is commonly due to an A to G point mutation at position 376 (A+ variant) or due to a mutation at this position combined with one of three other mutations (A- variant). G6PD deficiency is associated with oxidant-induced hemolytic anemia and increased oxidant stress, however the vascular consequences of G6PD deficiency are unknown. Since NADPH serves as an essential cofactor for several antioxidant enzymes and nitric oxide synthase, we hypothesized that G6PD deficiency would be associated with endothelial dysfunction. We genotyped the G6PD gene in healthy African Americans and identified 10 hemizygous men and 8 heterozygous women with the A376G mutation and 34 unaffected age-matched subjects (16 men and 18 women). As shown (Table) brachial artery flow-mediated dilation was impaired in men with the A+ variant compared to unaffected men. There was a trend for reduced red blood cell G6PD activity (assessed as time to NADPH-dependent dichlorophenol indophenol reduction) in the A+ variant men (64±39 min) compared to unaffected men (41±12 min, P = 0.096). Flow-mediated and nitroglycerin-mediated dilation and G6PD activity were similar in heterozygous and unaffected women. Thus, the A+ variant is associated with impairment in the biological activity of nitric oxide and may contribute to increased cardiovascular disease risk in African Americans.

Table. Effect of A376G Mutation on Vascular Function in African American Males

	Normal (n=16)	A+ Variant (n=10)	P
Flow-mediated dilation (%)	12.8±6.7	5.3±4.5	0.004
Flow-mediated dilation (mm)	0.53±0.21	0.17±0.21	0.001
Nitroglycerin-mediated dilation (%)	22.0±9.7	15.7±6.0	0.13

1079-132 Polymorphisms of the CETP, ABC, MTHFR, LPL, and PAI-1 Genes Predict Intermediate Endpoints but Not Angiographic Coronary Disease: Implications for Genetic Research

Benjamin D. Horne, Jeffrey L. Anderson, Joseph B. Muhlestein, Bryant M. Whiting, Tami L. Bair, Robert R. Pearson, John F. Carlquist, LDS Hospital, Salt Lake City, UT, University of Utah, Salt Lake City, UT

Background: Moderate-sized studies show that common functional variants of candidate genes predict intermediate phenotypes for coronary artery disease (CAD), but whether those results are an efficient surrogate for predicting clinical CAD is unknown. We studied a single nucleotide polymorphism (SNP) from each of five candidate genes as a predictor of intermediate (laboratory) and clinical endpoints (angiographic CAD).
Methods: Patients in the Registry of the Intermountain Heart Collaborative Study were genotyped for 5 SNPs: cholesteryl ester transfer protein (CETP) *Taq*IB (N=3,391), ATP-binding cassette (ABC) G596A (N=3,279), methylenetetrahydrofolate (MTHFR) C677T (N=731), lipoprotein lipase (LPL) *Hind*III (N=1,011), and plasminogen activator inhibitor (PAI)-1 4G/5G (N=1,246). Intermediate outcomes included high-density lipoprotein (HDL), triglycerides (TG), or total homocysteine (tHCY). Cases had 1-3 vessel CAD (≥70% stenosis); controls had angiographically normal coronaries. Adjustment was made for age, sex, and CAD risk factors.
Results: Average age was 64±12 years, 69% were male. CETP predicted HDL (mean: B1B1=34.8, B1B2=36.6, B2B2=38.4 mg/dL; $p=0.001$) but not CAD (B1: 75%, B2B2: 71%; $p=0.22$, odds ratio [OR]=1.15). ABC predicted HDL (mean: GG=36.5, GA=35.9, AA=38.0 mg/dL; $p=0.07$) but not CAD (GG: 74%, A: 75%; $p=0.65$, OR=1.04). MTHFR predicted tHCY (median: CC=13.1, CT=13.3, TT=14.4 µmol/L; $p=0.02$) but not CAD (C: 79%, TT: 77%; $p=0.66$, OR=0.88). LPL predicted TG (median: [--] 98, [-+] 124, [++] 134 mg/dL; $p=0.02$) but not CAD ([-] 78%, [++] 78%; $p=0.93$, OR=0.99). PAI-1 predicted TG (median: 4G4G=130, 4G5G=137, 5G5G=148 mg/dL; $p=0.002$) but not CAD (4G: 77%, 5G5G: 76%; $p=0.79$, OR=0.96).
Conclusions: Five SNPs predicted differences in risk-related lipids or tHCY, but not angiographic CAD. The discrepancies between intermediate and clinical phenotypes suggest that common polygenic factors modulate the clinical risk associated with candidate genes' susceptibility variants. Studies of haplotypes (rather than SNPs), multiple interacting genes, and associations to clinical (rather than intermediate) phenotypes may be necessary to unravel the complex genetic basis of CAD.

1079-133 Murine Cytomegalovirus Infection Increases Aortic Expression of Proatherosclerotic Genes

Mary Susan Burnett, Sarfraz A. Durrani, Motoyasu Saji, Cindy Gulick, Eric P. Hoffman, Stephen E. Epstein, MedStar Research Institute, Washington, DC, Children's National Medical Center, Washington, DC

Background: Results in several recent antibiotic trials raise questions as to the possible role of infections in cardiovascular disease. While these findings suggest that bacterial infections sensitive to the antibiotic administered may not contribute to an increase in cardiovascular events, these studies do not test the potential role of viral and non-sensitive bacterial infections in disease progression. Murine cytomegalovirus (MCMV) infection of apoE -/- mice increases atherosclerotic lesion size. In this study gene arrays were used to determine if MCMV infection produces atherogenic changes in aortic gene expression.
Methods: ApoE -/- mice were infected with 30,000 PFU of MCMV; a second group was given an uninfected cell supernatant. One week post infection, aortas were collected and pooled. Total RNA was extracted and reverse transcribed into double stranded cDNA. Labeled cRNA was hybridized to Affymetrix murine U74A chips. Data were analyzed using Microarray Suite software.
Results: MCMV infection changed aortic gene expression in the apoE -/- mice. Of the 100 genes that were differentially expressed, 25% are involved in inflammation/immune responses. Three genes, encoding MCP-1, IP-10 and MIG, are known to be proatherosclerotic and may predispose to plaque destabilization. These molecules are active in monocyte and T cell recruitment, and induce T cell adherence to endothelial cells. The ~10 fold increase in expression levels observed for these genes was confirmed in a separate group of animals, by western blotting, or TaqMan PCR.
Conclusions: In the apoE -/- aorta, MCMV infection significantly increases expression of three genes: MCP-1, IP-10, and MIG,known to play important roles in atherogenesis, and perhaps plaque destabilization. These infection-induced effects indicate a possible mechanism by which pathogens and the immune/inflammatory responses they elicit contribute to atherosclerotic disease initiation, progression, and precipitation of complicating clinical events. These results also emphasize that the negative results of macrolide antibiotic trials are not relevant to potential atherogenic effects of pathogens not sensitive to *these therapeutic agents*.

1079-134 Interactive Effect of the Glutathione S-Transferase Null Genotypes M1 and T1 and Cigarette Smoking on Coronary Artery Disease Risk and DNA Damage

Serena Masetti, Nicoletta Botto, Samantha Manfredi, Maria Giovanna Colombo, Antonio Rizza, Aldo Clerico, Andrea Biagini, Maria Grazia Andreassi, CNR, Institute of Clinical Physiology, G. Pasquinucci Hospital, Massa, Italy

Background: It has been suggested that cancer and altherosclerosis might share a common etiology. GSTS polymorphisms, which are associated with increased cancer risk, may be important in modulating susceptibility to CAD.
Aim: to evaluate Glutathione S-transferases (GSTs) null M1 and T1 (GSTM1 and GSTT1) genotypes as susceptibility genetic risk factors for coronary artery disease (CAD) and DNA damage.
Methods: We studied 430 patients who underwent coronary angiography, and examined the levels of DNA damage in 80 male patients by the micronucleus (MN) test, a sensitive assay for evaluating DNA damage.
Results: The frequencies of GSTs null genotypes in 308 patients with CAD were not significantly different from those of 122 patients without CAD. We observed, however, that GSTs null genotypes are associated with the occurrence of CAD among smokers (odds ratios (OR) and 95% confidence interval (CI) were OR= 2.2, CI 95%= 1.2-4.2, $p=0.01$; OR= 3.4, CI 95%= 1.6-7.1, $p=0.001$; OR= 4.0, CI 95%= 1.4-11.5, $p=0.01$, for smokers with null GSTM1, GSTT1 and both null genes as compared to never smokers with the present GSTs genotypes, respectively). There was a significant association between smoker null genotypes and both the number of stenosed vessels chi square=7.6, $p=0.05$; chi square =13.7, $p=0.0003$; chi square =10.0, $p=0.02$, for GSTM1, GSTT1 and both genes, respectively) and the Duke score ($p= 0.03$; $p=0.0002$; $p=0.004$, for GSTM1, GSTT1 and both genes, respectively) as compared to non-smokers patients expressing the genes.
MN levels were significantly higher in smokers null GSTs individuals as compared to smokers with present GSTs genes ($p=0.004$; $p=0.007$; $p=0.002$, for GSTM1, GSTT1 and both genes, respectively).
Conclusion: These observations suggest that GSTs-null genotypes strenghten the effect of smoking on CAD risk, likely by modulating the detoxification of genotoxic atherogens.