ROLE OF TLR4 POLYMORPHISMS IN INFLAMMATORY RESPONSES. IMPLICATIONS FOR UNSUCCESSFUL AND SUCCESSFUL AGEING

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Total burden of infections at various sites may affect the progression of Alzheimer's disease (AD) and atherosclerosis (CVD), the risk being modulated by host genotype. The role of lipopolysaccaride (LPS) receptor TLR4 is paradigmatic. It initiates the innate immune response against gram-negative bacteria and TLR4 single nucleotide polymorphisms (SNP), as Asp299Gly, suggested to attenuate receptor signalling, have been described. TLR4 Asp299Gly SNP shows a significant lower frequency in patients affected by AD or by myocardial infarction respect to controls, whereas centenarians show higher frequency. Thus, people genetically predisposed to develop a weak inflammatory activity, seems to have less chances to develop AD or CVD and, subsequently, live longer if they do not become affected by serious infectious diseases. To validate this hypothesis, the levels of pro-inflammatory cytokines, interleukin(IL)-6 and tumor necrosis factor(TNF9-α and anti-inflammatory cytokine IL-10, conversely involved in these age-related diseases and longevity, have been determined by ELISA in supernatants from whole blood assay of young health subjects after stimulation with subliminal doses of LPS from E.Coli. Besides, in these supernatants we also assessed by ELISA the levels of the inflammatory mediators eicosanoids (LTB4 and PGE2) produced by conversion of arachidonic acid for action of 5-lipoxygenase and cyclooxygenase. Samples, genotyped for Asp299Gly SNP, were challenged with LPS for 4, 24 and 48 hours. IL-6 and TFN-α levels were significantly lower in supernatants from carriers bearing TLR4 mutation, whereas IL-10 levels were significantly higher. Lower levels of LTB4 and PGE2 have been also observed in supernatants of Asp299Gly polymorphism carriers. So, these results clearly show that pathogen burden, by interacting with host genotype, determines the type and intensity of the immuno-inflammatory responses. Hence, this TLR4 SNP, which attenuates receptor signalling, enhances the risk of infections but decreases that of unsuccessful ageing as AD and CVD, by limiting inflammatory responses.

POLYMORPHISMS OF THE ECOSANOID ENZYMES IN SUCCESSFUL AND UNSUCCESSFUL AGEING. IMPLICATIONS FOR PHARMACOGENOMICS

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Recent studies have outlined crucial roles for prostaglandins and leukotrienes, in chronic inflammation including both atherosclerotic and senile plaques. In fact, PGE2 activates Matrix Metallo-proteinases whereas LTB4 is a chemoactractant for monocytes and activates the gene expression in inflammatory cells. COX-1 and COX-2 are the key enzymes in the conversion of arachidonic acid to prostaglandins and COX-2 is largely expressed in atherosclerotic plaques and in the Alzheimer's disease (AD) brain. LO enzymes catalyse the insertion of molecular oxygen into various positions in arachidonic acid and 5-LO is the initial key enzyme of the leukotriene pathway. The 5-LO enzyme has been described in endothelial cells as well as in neurons and in some glial cells throughout the cerebrum, basal ganglia and hippocampus. In the present study we have tested the hypothesis that anti-inflammatory variants of these genes confer genetic resistance to AD or infarction (AMI) and conversely favour longevity. So, we analyzed cohorts constituted by AD and AMI patients, age-related controls centenarians. The pro-inflammatory alleles of COX-2 and 5-LO were overrepresented both in AMI and AD patients and under-represented in centenarians whereas age-related controls displayed intermediate values. AD and AMI are multifactorial diseases, one cause is not sufficient to develop the disease, therefore AD and AMI might be the result of a cumulative effect which contributes with different timing to achieve a threshold where the chance to develop the diseases is very high. Difference in inflammatory status can contribute to draw a risk phenotype that does not allow to reach successful ageing. Hence, these studies are meant to detect and utilize a risk profile which allows early identification of individuals susceptible to disease and design of the right dose for a desired effect, a pharmacogenomic approach for age-related diseases, i.e. a preventive treatment with specific inhibitors of eicosanoids or their enzymes.

GENETICS OF INFLAMMATION IN ATHEROSCLEROSIS AND LONGEVITY

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The inflammatory response, set by tissues in response to injury elicited by trauma or infection, is a complex network of molecular and cellular interactions that facilitates a return to physiological homeostasis and tissue repair. The individual response is also determined by gene variability. Ageing is accompanied by chronic low-grade inflammation state clearly showed by 2-4-fold increase in serum levels of inflammatory mediators. A wide range of factors has been claimed to contribute to this state; however, the most important role seems to be played by the chronic antigenic stress which affects immune system thorough out life with a progressive activation of macrophages and related cells. This pro-inflammatory status, interacting with the genetic background, potentially triggers the onset of age-related inflammatory diseases as atherosclerosis. Thus, the analysis of polymorphisms of the genes that are key nodes of the natural immunity response might clarify the patho-physiology of atherosclerosis. On the other hand, centenarians are characterized by marked delay or escape from age-associated diseases that, on average, cause mortality at earlier ages. In addition, centenarian offspring have increased likelihood of surviving to 100 years and show a reduced prevalence of age-associated diseases, as cardiovascular disease (CVD) and less prevalence of cardiovascular risk factors. So, genes involved in CVD may play an opposite role in human longevity. Thus, the model of centenarians can be used to understand the role of these genes in successful and unsuccessful ageing. Accordingly, we discuss our results showing the frequencies of pro-inflammatory alleles were significantly higher in patients affected by infarction and lower in centenarians whereas age-related controls displayed intermediate values. These findings point to a strong relationship between the genetics of inflammation, successful ageing and the control of cardiovascular disease.

SERUM PROTEIN LINKED N-GLYCOMIC CHANGES DURING HUMAN AGEING: A NEW FUNCTIONAL AGEING BIOMARKER?

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Background: Glycosylation is not random but is highly reproducible in a given physiolocal state. It is well accepted that the N-linked oligosaccharides of glycoproteins play important biological roles by influencing the functions of glycoproteins. Significant changes in cellular processes, such as ageing and ageing-related diseases, may be expected to result in alterations to the glycan profiles of secreted glycoproteins. N-glycan profiling is expected to be used as an ageing biomarker predicting the condition of human health.

Methods: DSA-FACE based N-glycan analysis system is a high throughput technology platform and is designed to detect N-glycan profiles from glycoproteins in serum or other body fluids. 219 serum or plasma of the healthy people in different age groups and 129 plasma of the centenarian were used by N-glycan analysis.

Results: The levels of three N-glycan sugar structures (NG0A2F, NG0A2FB and NA2F) in blood are altered with ageing. There is an increasing abundance of agalactosylation (NG0A2F, NG0A2FB) and a decreasing abundance of core fucose digalatosylated biantennary (NA2F). This demonstrates that the alteration of N-glycosylation is associated with ageing processes.

Conclusion: The measurement of N-glycan level could provide a noninvasive marker for man's health condition or the forecasting of disease progression upon ageing, and for efficacy of anti-ageing treatment or following up the patient´s condition after therapy.

+647 A/C AND +1245 A/G MT1A POLYMORPHISMS AND ATHEROSCLEROSIS: ZINCAGE STUDY

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Low zinc intake, reduced blood zinc concentrations and depleted enzyme antioxidant activity may be a risk factors for atherosclerosis (AT).
Metallothioneins (MT) are zinc binding proteins regulating zinc homeostasis and protecting from oxidative stress, which plays a key role in AT development .
-209 MT2A polymorphism is involved in ischaemic cardiomiopathy in diabetes. No study exists on MT1 A polymorphisms and AT.
The present investigation analyses +647 A/C and +1245 A/G MT1A polymorphisms in 403 old healthy subjects and 340 AT patients from Italy, Greece, France.
Dietary zinc intake [expressed as zinc score (Frequency x Quantity x Zinc content in the food) calculated for each recruited subject as the sum of all estimated zinc intakes derived from all food items], was higher in France and lowest in Greece ($p<0.001$). Zinc score was no different between AT patients and old controls.
Concomitantly, zinc status (erythrocyte and plasma concentration) in healthy elderly was higher in France lower in Greece, with intermediate values in Italy ($p<0.01$). Decreased plasma zinc was only observed in AT Italian patients.
A multiple logistic regression analysis revealed that, MT1 A +647 C+ genotype and MT1A +1245 G+ genotype were significant genetic risk factors for AT (odds ratio=2.85, P=0.007; odds ratio=3.57, p=0.003 respectively) in Greece, but not in Italy or France. However, considering a subgroup of subjects (the 66th percentile of zinc score) in Italian sample, higher 647 C+ and 1245 G+ genotypic frequencies were present in AT patients ($p<0.05$).
Moreover, +647 C+ and +1245 G+ MT1A genotypes are related to altered glutathione peroxidase and catalase activity in Greece, whereas in Italy enzyme antioxidant activity was only influenced by +1245 MT1A polymorphism. In conclusion, +647 and +1245 MT1A polymorphisms are associated with AT in countries with lower zinc intake and zinc status.

ASSOCIATION BETWEEN PLATELET ENDOTHELIAL CELLULAR ADHESION MOLECULE 1 (PECAM-1/CD31) POLYMORPHISMS AND ATHEROSCLEROSIS: RESULTS OF A STUDY PERFORMED IN PATIENTS FROM NORTH ITALY

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Adhesion of circulating cells to the arterial surface is among the first detectable events in atherogenesis. Cellular adhesion molecules mediate cell recruitment and their transendothelial migration. Platelet endothelial cellular adhesion molecule-1 (PECAM-1/CD31), involved in this migration, has been associated with atherosclerosis. Studies have investigated an association between coronary artery disease (CAD) and single nucleotide polymorphisms (SNPs) located in functionally important domains of the PECAM-1/CD31 gene, with contrasting results. In particular, in our previous study, the frequency of the Gly670Arg SNP was significantly higher, although borderline, in patients with myocardial infarction, whereas the frequencies of other two SNPs (Leu125Val and Ser563Asn) were not significantly different between patients and controls. To check the validity of our results, we have analysed the distribution of these SNPs in another group of patients and controls of North Italy. Besides, we also analyzed a novel functional variant recently identified in the 5' UTR of the PECAM-1/CD31 gene (53 G>A). So, we analysed by PCR-SSP the G53A, Val125Leu, Asn563Ser and Gly670Arg SNPs of PECAM-1/CD31 in 320 patients affected by CAD and 119 healthy controls from North Italy. Our results demonstrate that were not significant differences between patients and controls for Val125Leu, Asn563Ser and Gly670Arg SNP, also analyzing data according to gender. As regards the 53G/A SNP, the analysis of genotype and allele frequencies demonstrate a significant difference between patients affected by CAD and healthy controls ($p=0.0040$ and respectively $p=0.0016$). In conclusion, in our study, the 53 G/A SNP seems to be involved in CAD onset. So present and previous data suggest that PECAM-1/CD31 plays a role in unsuccessful ageing development.

RESISTANCE TO ACUTE OXIDATIVE DAMAGE INDUCED BY RENAL ISCHEMIA IN MOUSE MODELS OF THE SEGMENTAL PROGERIA COCKAYNE SYNDROME

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Oxidation of protein, lipids and DNA is thought to underlie the aging process. Extended longevity in a number of model organisms correlates with resistance to oxidative stress, while hypersensitivity to oxidative stress may underlie segmental progeroid disorders including Cockayne syndrome, a congenital nucleotide excision DNA repair (NER) deficiency. We recently reported the surprising finding that short-lived NER mouse models share physiological properties with long-lived calorie restricted or endocrine-deficient mice, including reduced temperature, reduced blood glucose and reduced serum insulin-like growth factor-1 (van de Ven et al. PLoS Gen. 2006). Because these phenotypes correlate with and may underlie acute oxidative stress resistance in long-lived animals, a paradox is suggested in which DNA repair deficiency can cause, on the one hand, oxidative stress hypersensitivity resulting in progeria, and on the other, oxidative stress resistance. Here, we tested acute oxidative stress sensitivity of NER-deficient mice using an in vivo model of oxidative stress-related injury, surgically-induced renal ischemia reperfusion. We found Cockayne syndrome mouse models protected from renal ischemic injury relative to wildtype animals. Renal failure-related mortality was significantly reduced from 70% in wildtype mice to 40% in *Csb*$^{-/-}$ mice, kidney function was improved, and proliferation was significantly higher in kidneys in the regenerative phase following the injury. Upregulation of cytoprotective proteins including hemoxygenase-1 and increased glucose tolerance correlated with increased regeneration following injury. Protection from ischemic injury was associated specifically with mutants defective in the transcription-coupled subpathway (*Csb*$^{-/-}$, *Csa*$^{-/-}$ and *Xpa*$^{-/-}$) but not the global genome subpathway (*Xpc*$^{-/-}$) of NER. These genetic data suggest that specific types of unrepaired endogenous DNA lesions can induce hormetic effects against oxidative stress via mechanisms common to lifespan-extending calorie restriction and endocrine-deficient dwarfism. Hormesis is a recognized phenomenon in which toxic substances have beneficial effects at low doses, but its extension to genotoxic agents remains controversial.

EPIGENETIC CHANGES OF THE *dfna5* GENE REGION DURING DIFFERENTIATION AND AGEING

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Epigenetic chromatin structural alterations affect gene expression during development and ageing. This prompted us to investigate changes in histone modifications that may occur to specific age-related gene targets during these two processes. For this purpose, we used isolated monocytes from different donor age groups (cord blood, young, elderly) induced to differentiate *in vitro* into dendritic cells. Microarray gene expression analysis, showed that *dfna5* (deafness autosomal dominant 5) gene expression levels changed during differentiation and also amongst the different age groups. This finding, coupled with the fact that this gene was observed to be expressed in fetal, but not in adult, mouse cochlea, strongly indicated an association of this gene's expression levels with development and aging. So as to verify the microarray results, we checked the expression levels of *dfna5* and its transcription rate. The results showed that its expression levels and transcription rate were very low in monocytes but highly increased in differentiated dendritic cells. With respect to the different age groups, we found that cord blood dendritic cells express more *dfna5* than dendritic cells of young donors. However, *dfna5* expression levels increased significantly again in the samples from the elderly donors. We then proceeded to investigate the possible epigenetic changes that may occur in the *dfna5* gene region using ChIP analysis. We found increased histone acetylation in the dendritic cells as compared to the monocytes, but no age-related differences. On the other hand, age-related differences were found in the histone methylation levels. The differences found in the histone modification status of this gene correspond to the observed expression levels of *dfna5*. Future studies will include the analysis of a greater area of the chromatin regions surrounding this gene so as to ascertain whether further upstream and downstream chromatin remodeling events influence its gene expression levels.

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ZINC AND UNSUCCESSFUL AGEING

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Life long antigenic burden determines a condition of chronic inflammation, with increased lymphocyte activation and pro-inflammatory cytokines production. A large number of studies have documented changes in Zn metabolism in experimental animal models of acute and chronic inflammation and in human chronic inflammatory conditions. In particular, modification of zinc plasma concentration, as well as intracellular disturbance of antioxidant intracellular pathways, have been found in ageing and in some age-related diseases. Zinc deficiency is diffused in aged individuals in order to avoid meat and other high zinc-content foods due to fear of cholesterol. Rather, they increase consumption of refined wheat products that lack of zinc and other critical nutrients in consequence of refining process. On the other hand, plasma zinc concentration is influenced by pro-inflammatory cytokines (IL-6 and TNF-α) and by Metallothioneins (MT) homeostasis, which is in turn affected by pro-inflammatory cytokines. MT increase in ageing and chronic inflammation allowing a continuous sequestration of intracellular zinc with subsequent low zinc ion availability against stressor agents and inflammation. This phenomenon leads to an impaired inflammatory/immune response in elderly. So, it is not so surprising that zinc deficiency is constantly observed in chronic inflammation, such as in old individuals. On the other hand, cytokine genes are highly polymorphic and some of these polymorphisms are associated with atherosclerosis and diabetes type II. Therefore, zinc turnover, via MT homeostasis, in individuals genetically predisposed to a disregulation of the inflammatory/immune response may play a crucial role in causing possible adverse events leading to unsuccessful ageing and age related disease development.