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Viewpoint

Is it time for a new approach in cardiovascular risk assessment?

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FROM CONVENTIONAL RISK FACTORS TO THE CLUSTERING OF VARIOUS FACTORS

Prospective epidemiologic studies have established risk factors for atherosclerotic cardiovascular diseases, including: advanced age, smoking, obesity, diabetes or glucose intolerance, elevated blood lipids, hypertension and sedentary life habits. Many of these factors are closely related to modern lifestyles and can be modified. They have been used for the risk estimation and the planning of preventive measures *(1, 2)*.

In addition to the commonly used classic risk factors, there is evidence suggesting the existence of novel risk factors, including: inflammation, chronic latent infections e.g. *cytomegalovirus* or *Helicobacter pylori* infection, hypercoagulability, impaired fibrinolysis, increased platelet reactivity, enhanced oxydative stress, elevated blood homocysteine levels and lipoprotein particles such as lipoprotein (a) and small dense low-density lipoprotein cholesterol *(3)*. Depression and complex socioeconomic factors have also been recognized as factors strongly influencing the prognosis in cardiovascular diseases *(3, 4)*. These novel factors might be useful in stratifying cardiovascular disease severity and prognosis more accurately. This may be important especially in cases free of conventional risk factors, as it has been shown that up to one third of first coronary events occur among individuals without conventional risk factors *(5)*. In addition, the same risk factors may not have the same causal effect in different ethnic and population groups *(6)*.

Moreover, there is an awareness of the fact that risk factors tend to appear in a cluster, linked to each other. For example, the metabolic or insulin resistance syndrome has been identified on the basis of the clustering of multiple cardiovascular risk factors *(7)*. Similarly, end-stage renal disease has been recognized as a multirisk condition, characterized by an unexpectedly high prevalence of cardiovascular diseases *(8)*.

Taking all these into consideration, it is not surprising that the preparation of cardiovascular risk estimation tables and guidelines becomes more and more difficult *(9)*. Two opposite, but also complementary claims can be observed: a need for a more comprehensive and, at the same time, a more individually based approach in cardiovascular risk assessment. In particular, the problem is how to interpret the results of the basic research and clinical and epidemiologic studies to be specifically oriented towards conditions of an individual. Evidence from many fields of research indicates that it is time for a new approach in cardiovascular disease research and risk assessment.

THE HUMAN GENOME PROJECT, GENOMICS, PROTEOMICS, PERSONALISED MEDICINE

The most important question regarding prevention in cardiovascular diseases is how to determine to what extent they are inheritable and to what extent environmental factors contribute to their expression *(10)*. The recent breakthrough in the Human Genome Project, tightly linked to the progress in molecular biology techniques, has been expected to provide insight into the genetic background of cardiovascular and other complex common diseases *(11)*. However, high expectations were replaced by overwhelming skepticism about the near-term applications of genomics in health care and common disease prevention *(12)*. Gene profiling assays so far have not provided benefits in cardiovascular risk prediction beyond the current approach, by using conventional risk factors *(10, 11, 12, 13)*. It has been shown that there is no specific disease susceptibility genotype, just numerous genetic variants which account simultaneously for many age-related phenotypes. That means that the individual variant, important in the particular molecular pathway, is not sufficient to cause clinical expression of a disease. Instead, common diseases in the population result from the complex interactions among multiple genetic variants and environmental risk factors. Thus, genetic and environmental factors are shared between clinically related disorders *(13)*.

Yet, a more positive note is that in common disease prevention, practical application of genomics is going to assume more realistic dimensions. Genetic information is now used only in addition to public health interventions, not as the task of the priority. This is because the major environmental factors, known to influence the frequency of common diseases and the mortality rate in the population, such as: cigarette smoking, unhealthy diet, physical inactivity, excessive alcohol use, infections, injuries and exposure to toxins, were proved to be to a great degree modifible. To assess whether interventions based on genetics might add value in disease prevention to the existing approach, by implementing public health measures, information is required on: the prevalence of high-risk genetic variants in the given population, the strength of the association between these variants and a disease of interest by means of a population perspective, and modification of risk by other genetic and environmental factors *(12)*.

Personalised medicine continues to be the desired goal of health care in general and, in particular, of preventive medicine *(14)*. Realization of this idea is closely related to the problem of conversion from basic science to clinical applications. Advances in genomics and proteomics give promise that it could be possible to cover the distance from discovering new knowledge to provide better health care to individuals and to the population as a whole *(12, 14)*. The greatest effects are expected in personalised nutrition, specifically aimed at maintaining the health of an individual and prevention of diseases, as well as in customizing therapy, by maximizing the effectiveness of drugs and minimizing their side effects. The challenges also lie in the detection of persons with early deviations, long before biochemical and clinical signs of a disease become clearly measurable *(14, 15)*. In addition, the availability of computer programs in practical application in medicine is expected to allow the choice of such diagnostic and therapeutic protocols which could meet the specific needs of an individual with the priorities. Accomplishing the goal of personalised medicine will also require systematic knowledge integration and transdisciplinary collaboration, as well as efforts in the development of a health care policy and services *(12, 14, 16)*.

COMMON BACKGROUND FOR ATHEROSCLEROSIS AND OTHER AGING DISORDERS, AGING THEORIES

Two lines of evidence, one showing atherosclerosis as an inflammatory disease and the other indicating chronic inflammation as the main driving force underlying the processes of aging, seem to converge into one unique concept, putting atherosclerosis into the same pathogenetic background with other common aging disorders.

Atherosclerosis was first considered a lipid-storage arterial disease. Subsequently, it has been established as a chronic inflammatory disease. In particular, the role of innate immunity has been confirmed equally in all stages of plaque development, from initiation, progression, to thrombotic complications *(17, 18)*. Also, it has been found that low-grade inflammation, indicated by increased serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6), may predict the risk of acute myocardial infarction independently of traditional risk factors *(19, 20)*. Besides inflammatory mediators produced locally, within the affected vascular wall, the production of mediators at other sites of infection and inflammation in the body, if released in the circulation, may contribute to the progression of atherosclerosis *(21)*. Furthermore, recent studies on genetics provide evidence that testing on the polymorphism of proinflammatory genes may add information to the cardiovascular risk profile. Included genes are those coding for: proinflammatory cytokines (TNF-a, IL-1 and IL-6), toll-like receptors, known to initiate the innate immune response to bacterial lipopolysaccharide, and lipid mediators cyclooxygenase-1 and -2 and lipooxygenase-5 *(10, 21, 22)*.

Numerous aging theories have been constructed, indicating the complex and largely unknown process of aging. Former theories were monistic in nature, assuming one key factor responsible for the aging. They can generally be classified in two categories: program theories and error (mutation) theories. According to program theories, aging is considered as a genetically determined process. The error theories imply the accumulation of the deleterious changes in cells and tissues, leading to mutations, regulation disorders and clinically obvious dysfunctions *(23)*. New theories, in contrast, are multifactorial and tend to reflect the complex and highly integrated nature of the human body *(21, 23)*. Thus, according to

»the network theory of aging«, aging is controlled by the network of cellular and molecular defense mechanisms, involving: DNA repair mechanisms, heat shock protein production, anti-oxidant system, apoptosis, cell senescence, scavenging of damaged cells and replacement of dead cells by stem cells. At the systems level, mechanisms of control are represented by immune and inflammatory responses, stress response and neuroendocrine responses *(24)*. A major assumption of this theory is that the variable longevity of individuals in the population can be explained by subtle differences in the efficiency of their network of defense mechanisms. Based on this assumption, many age-related changes of the immune parameters could be explained as a result of an adaptation of the immune system, with time, to the deteriorative environmental agents (the process termed »immunosenescence«) *(25)*. Following a similar assumption, age-related changes in metabolic pathways and in endocrine functions, especially those concerning the hypothalamus-pituitary-adrenal (HPA) stress axis, were also explained as the time-dependent remodelling process (the paradigm named »the remodelling theory of aging«) *(24, 26)*.

Evidence suggests that these three systems, the immune, the metabolic and the endocrine, are connected and capable of influencing each other during the age-dependent remodelling *(21, 26, 27, 28)*. Chronic inflammation has been recognized as that mechanism which links changes in these systems together, leading to the development of the main aging diseases, including: atherosclerosis, cancer, Alzheimeržs disease, diabetes, osteoporosis and osteoarthritis *(21, 29)*. In favor of such an assumption, factors previously already found to contribute to the state of chronic inflammation are: insulin resistance and central body fat distribution (reflecting the remodelling of the metabolic pathways), decreased production of sex steroids and the HPA stress axis over-activation (due to the remodelling of the neuroendocrine system), existing sites of chronic inflammation (such as atherosclerotic vessels) and the chronic activation of the innate immune system (due to immunosenescence). In addition, the genetically determined capacity of inflammatory mediator production and, reversely, the capacity of anti-inflammatory (defence) network, might contribute to the fine tuning of the inflammatory responses *(21, 29)*.

Epigenetic regulation of gene expression, intrinsic and environmental signals, intra-individial variability, self-sustaining (stochastic) nature of aging mechanisms

An important assumption emerges from the comprehensive theories of aging, mentioned above. That is, inflammation and other age-related changes of cell microenvironment can cause changes in gene expression and proteome profiling in the local tissue environment *(29)*. There is evidence suggesting that chemicals, produced during metabolic and inflammatory processes, e.g. reactive oxygen species, may interfere with »normal« DNA transcriptional and repair processes and with intra- and inter-cellular signaling *(27, 30, 31)*.

Two important consequences may result from this assumption. One is the fact that inner physiological perturbations, by themselves, can be included in regulation processes. This may further imply the self-sustaining, stochastic nature of the aging mechanisms *(31)*. In favour of this, efforts over the past few years have been focused on molecular mechanisms that mediate epigenetic mechanisms of control of gene expression. As a potentially most important mechanism, change in DNA methylation has been proposed *(32)*. This mechanism, arising from the age-related disturbation in certain nutritional and cellular metabolic pathways, seems to be capable of promoting genetic changes in somatic cells. This may initiate a sequence of reactions, leading step by step to the development of cancer and other aging diseases *(32, 33)*. In addition, it is supposed that epigenetic mechanisms of the control of gene activity represent the main way through which an organism responds to external environmental signals. In this way, it is difficult to strongly divide intrinsic and environmental signals *(32)*.

Another important consequence of locally mediated changes in gene expression and proteome profiling, emerging from this discussion, is the difference in the rate of aging among different organs and systems of the body. This phenomenon really accompanies aging and is termed »the intra-individual variability« *(34)*. Based on these facts, the study of aging is connected with substantial difficulties.

Population statistics, mortality curves, inter-individual variability, complex systems

For the purpose of vital statistics data recording, the monotonic hazard functions are usually employed, describing the age dependence of mortality *(35, 36, 37)*. This type of approach can also be used to gain insights into the link between the aging processes and mortality. According to Gompertz' law, the most widely used theoretical model of mortality kinetics, progressive deterioration in the vitality (an overall index of physiologic capacity) is proposed. However, this model does not fit the empirical data in all applications. Exactly, discrepancy appears when data of people in advanced age are considered, reflecting inter-individual differences in the age-dependent risk of death. In such a situation, the end of the mortality curve begins a slow, downward direction instead of continuing its positive exponential course, reflecting the positive selection of living longer, more healthy individuals and the exhaustion of high-risk subgroups. In general, data concerning heterogeneity must be included in any model aimed at interpreting the link between aging and mortality correctly. It is supposed that the variability of biological parameters, among individuals of the population, visible especially in the elderly, can be a result of a dynamic interplay between biological factors and the environment. However, results of studies on laboratory-housing animals suggest that it is impossible

to eliminate heterogeneity completely, even in the case of extremely homogeneous conditions, regarding both genetical and environmental factors. In order to find an appropriate description of this phenomenon, an entirely new approach in the research of aging was entered *(35, 36, 37)*.

Thus, if human beings are considered as nonlinear complex systems, their age-related changes can easily be explained from that point of view. This means that even small differences among subjects at the time of birth will evolve, fluctuating with time, progressively enlarging the variability of their characteristics, including life-span *(31, 35, 36, 38, 39)*. This may be a result of the fundamental property of complex systems, known as »dependency on the starting conditions« *(38, 39)*. Other major features of complex systems which may contribute to a large diversity of aging phenotypes include their relative stability on minor changes in the environment, but great instability in the case of larger shocks, resulting in many ways in which the response can be manifested *(35, 38, 39)*. The principle of »locality«, another major feature of complex systems, means a process composed of a variety of strictly spatially organized mechanisms of cell-cell interactions, taking place locally, in tissues. This may be a basis for the intra-individual variability, described in the previous chapter *(31, 38)*.

In addition, complex systems show characteristics of an »emergent behavior«, meaning that a global behavior of the system as a whole cannot be understood on the basis of the knowledge of the behavior of its particular components *(31, 35, 38, 39)*. Such approach allows new strategies to be used in building mathematical models of aging, those from the perspective of systems biology science *(31, 40, 41)*. To find out the ways components of the system are linked to each other, forming functionally integrated units, a relatively large number of parameters will be required. The challenge will be to develop such mathematical models which will be able to integrate numerous concepts on aging into a unique model *(31)*. Attempts to study aging from the perspective of complex systems have already been done. In particular, findings are available suggesting a decrease of complexity with age *(42)*. These findings give the hope that, by studying aging from the point of view of complex systems, substantial new contributions could be obtained.

Aging and systems biology, bioinformatics, decision making processes in health care

Current theories of aging, mentioned above, emphasize aging as complex traits resulting from the interaction of genetics, environment and stochasticity. It is now obvious that there is a need for more comprehensive, systems approaches in studying aging. Therefore, studing humans is more advantageous than previous studies, prepared on animal models, owing to the high complexity of the human body. In particular, studing humans shows the benefit due to the influence of the social environment, dominated by cultural and anthropological habits, on characteristics of different population groups *(29, 31)*. A large contribution to the knowledge on aging has been obtained from comparative studies on centenarians, a specific population group characterized by healthy aging and extreme longevity *(26, 29, 43)*.

The relatively old idea of systems biology is now placed in the field of bioinformatics, linking it to the ability to collect experimental data on-line *(40, 41)*. The managing of a large database is proposed when building computer models. To achieve the goal of implementing systems biology methods in practical medicine, the development of advanced software and analytical mathematics should be an ongoing process *(31, 40, 41)*. In addition, a better understanding of human mental and reasoning processes will be necessary. Such attempts have been made in decision making processes in health care. The role of more humanistic strategies, such as visual cognition, pattern perception and intuitive processing, has been addressed *(44)*.

Systems biology approaches allow many unexpected results and hidden information to be obtained, otherwise not apparent from traditional approaches, by using phenomenological descriptions of a few data *(31)*. In general, computer modelling technics can be divided into two categories: bottom-up and top-down approaches. The top-down approach starts with a system as a whole and disects it into functional and computable entities. A description of higher levels of biological organization, such as cells arrangements in tissues and spatial representation of supply networks, such as the circulatory system and the bronchial tree of the lung, becomes visible by using this approach. In this way, fractal properties of biological structures (self-similarities, or re-occuring of basic shapes in filling up the space) have been found. Such spatial organization seems to be maximally space-saving and optimizing in terms of energy dissipation *(31)*.

In the bottom-up type of modelling, data on basic entities, such as genes and proteins, are integrated in order to define patterns in functional (time-scalled) organization. Methodological tools such as Multivariant analysis and Data-Mining, an advanced statistical technique, are required, when trying to generalize fundamental knowledge on individual parts and identify the key pathways and their connections. By using systems biology approaches, recent advances in human genome and proteome mapping projects have started to provide valuable contributions in our understanding of the biology of aging. For example, early results confirm certain topology in gene regulation pathways, meaning that a few genes have a higher number of connections, than the rest, and are able to receive and direct the signals of many genes. These genes, being constantly up- or down – regulated, seem to be more tightly associated with the aging process. They are usually involved in cell cycling and cell signaling, leading to the cellular senescence and apoptosis *(31)*.

Finally, multiscale, spatio-temporal models have recently been suggested, allowing molecular processes to

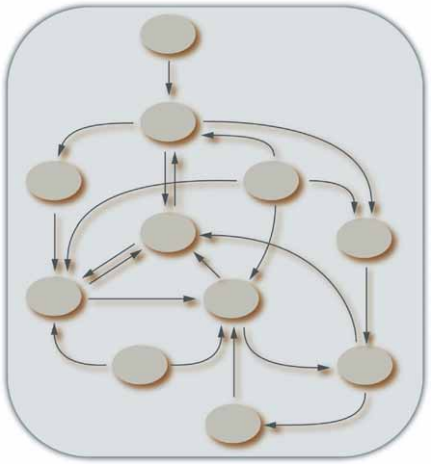

Figure 1. *»The pattern of disorders« or »the networked reaction«*

be represented in a line with structural organization. Such models are expected to cope best with the principles of aging, as aging phenotypes manifest on a systems level, but emerging from networked processes, regulated across different levels of biological organization *(31)*.

Is it time for a new approach in cardiovascular risk assessment?

It is now obvious that many phenomena in human pathophysiology, especially those concerning aging, cannot be explained by studying the impact of a single variable, or even a few of them. Because of many limitation of the reductionistic approach, still widely used in dealing with medical problems, the systems biology approach has got the chance to emerge. An increasing interest in the systems theory approach is also linked to the progress of science in many fields and advances in computer technology and applications as well. All necessary preconditions for its wider implementation in medicine have not yet been achieved. Scientists, engaged in aging research, emphasize two main obstacles in trying to study aging phenomena from the perspective of the systems-level approach. These are: an insufficient knowledge about determinants of the aging process and, consequently, the lack of standardized biomarkers of aging *(36, 37)*.

We assume that terms such as »biomarkers«, or »determinants«, represent elements of the reductionistic way of thinking, which sustains our sense of »cause and effect« when dealing with biological phenomena *(38, 39, 41)*. Therefore, it is most important that we change our way of thinking. We should direct our interests towards the behavior of the whole system, by pointing out typical »patterns of disorders« and their changes with time *(31, 38, 39)* (Figure 1, 2). Advanced »software« will be crucial in achieving this aim, allowing the management of a large set of data. Data of different types, both descriptional and numerical, clinical, biochemical, or those obtained by sophisticated laboratory technics, could be of interest. Mathematical models cannot incorporate all data available. Instead, computer modelling has to be directed on selected properties of the pathophysiologic process under investigation. By using »Data-Mining«, or some other advanced analytical technics, it will be possible to achieve »natural« clustering of data to become visible. Use of prior knowledge will be necessary, to complement pure statistical and clustering techniques *(31)*.

In order to make predictions about future adverse cardiovascular events, computerized patient record systems have to be employed, to allow collecting data prospectively. We suggest the methodology of defining »patterns of disorders«, in the population of interest, in relation to years of prediction (Figure 2). Even when considering a huge heterogeneity among individuals in the population, the definition of such patterns seems to be possible, as complex systems show an ability to occupy only a limited number of specific states *(38, 39)*. Conditions of any individual patient can then be compared with a set of patterns, previously obtained by computation of data on the population as a whole. Moreover, an individual risk could be more accurately fitted by using additional data, such as data on family history of a disease, subjective psychological profile, or intentionally designed genetical tests. The earlier we take the first steps, the faster we can move on the implementation of the systems biology approaches in medical practice.

The Data-Mining technic was used to analyse a large set of data. A cluster of data was obtained and explained in light of existing knowledge. In this way, a functional pathway, or »the pattern of disorders«, was constructed. *(A scheme was taken out from our original work on hyper-*

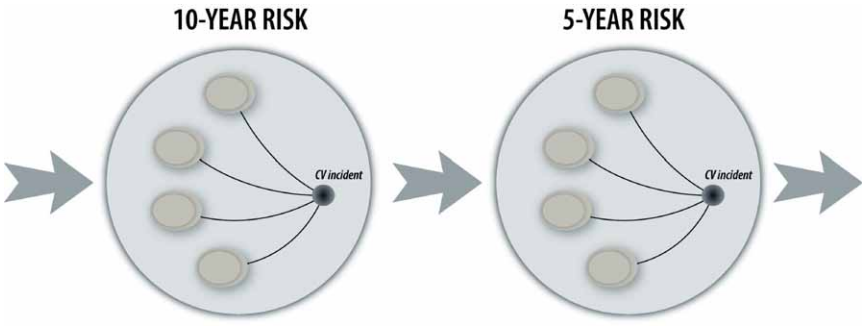

Figure 2. *Model of prediction of future cardiovascular incident (CV), from the perspective of complex systems science*

homocysteinemia. It is visible that some components in the network are more important, by having more connections, than others).

A limited number of »patterns of disorders« can be identified in the population. Changes in patterns, with time, can be used in cardiovascular risk assessment. By comparing conditions of an individual with a set of »patterns«, an individual risk can be estimated.

REFERENCES

1. LERNER D J, KANNEL W B 1986 Patterns of coronary heart disease morbidity and mortality in the sexes : A 26-year follow-up of the Framingham population. *Am Heart J February:* 383–390
2. COOPER J A, MILLER G J, HUMP HRIES S E 2005 A comparison of the PROCAM and Fraingham point-scoring systems for estimation of individual risk of coronary heart disease in the Second Northwick Park Heart Study. *Atherosclerosis 181:* 93–100
3. KULLO I J, GAU G T, TAJIK A J 2000 Novel risk factors for atherosclerosis. *Mayo Clinic Proc 75:* 369–380
4. ENGSTROM G, JERNTROP I, PESSAH-RASMUSSEN H, HEDBLAD B, BERGLUND G, JANZON L 2001 Geographic distribution of stroke incidence within an urban population: relations to socioeconomic circumstances and prevalence of cardiovascular risk factors. *Stroke 32(5):* 1098–103
5. HELLER R F, CHINN S, PEDOE H D, ROSE G 1984 How well can we predict coronary heart disease? Findings in the United Kingdom Heart Disease Prevention Project. *BMJ 288:* 1409–1411
6. REDDY K S, YUSUF S 1998 Emerging epidemic of cardiovascular disease in developing countries. *Circulation 97:* 596–601
7. MEIGS J B, D'AGOSTINO R B, WILSON P W F, CUPPLES L A, NATHAN D M, SINGER D E 1997 Risk variable clustering in the insulin resistance syndrome. *Diabetes 46:* 1594–1600
8. YOA Q, LINDHOM B, STENVINKEL P 2004 Inflammation as a cause of malnutrition, atherosclerotic cardiovascular disease, and poor outcome in hemodialysis patients. *Hemodial Int 8:* 118–129
9. INTERNATIONAL ATHEROSCLEROSIS SOCIETY 2003 Harmonized Clinical Guidelines on Prevention of Atherosclerotic Vascular Disease: Executive summary
10. CASAS J P, COOPER J, MILLER G J, HINGORANI A D, HUMPHRIES S E 2006 Investigating the genetic determinants of cardiovascular disease using candidate genes and meta-analysis of association studies. *Ann Human Genetics 70:* 145–169
11. YANG Q, KHOURY M J, FRIEDMAN J M, LITTLE J, FLANDERS W D 2005. How many genes underlie the occurrence of common complex diseases in the population. *Int J Epidemiol 34(5):* 1129–1137
12. KHOURY M J, GWINN M, BURKE W, BOWEN S, ZIMMERN R 2007 Will genomics widen or help heal the schism between medicine and public health. *Am J Prev Med 33(4):* 310–317
13. BECKER K G 2003 The common variants/multiple disease hypothesis of common complex genetic disorders. *Medical hypotheses 62(2):* 309–317
14. ASPINALL M G, HAMERMESH R G 2007 Realizing the promise of personalized medicine. Harvard Business Review, October, p 109–117
15. ORDOVAS J M, MOOSER V 2004 Nutrigenomics and nutrigenetics. *Curr Opin Lipidol 15(2):* 101–108
16. ZVAROVA J, STUDENY M 1997 Information theoretical approach to constitutions and reduction of medical data. *Int J Med Informatics 45:* 65–74
17. WICK G, SCHETT G, AMBERGER A, KLEINDIENST R, XU Q 1995 In atherosclerosis an immunologically mediated disease. *Immunol Today 16(1):* 27–33
18. YOKOTA T, HANSSON G K 1995 Immunological mechanisms in atherosclerosis. *J Int Med 218:* 479–489
19. RIDKER P M, RIFAI N, STAMPFER M J, HENNEKENS C H 2000 Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation 18:* 1767–1772
20. KULLER L H, TRACY R P, SHATEN J, MEILAHN E N 1996 Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol 144:* 537–47
21. VASTO S, CANDORE G, BALISTRERI C R, CARUSO M, COLONA-ROMANO G, GRIMALDI M P, LISTI F, NUZZO D, LIO D, CARUSO C 2007 Inflammatory networks in ageing, age-related diseases and longevity. *Mech Age Dev128:* 83–91
22. GRIMALDI M P, VASTO S, BALISTRERI C R, DI CARLO D, CARUSO M, INCALCATWRRA E, LIO D, CARUSO C, CANDORE G 2007 Genetics of inflammation in age-related atherosclerosis. *Ann N Y Acad Sci 1100:* 123–131
23. SEMSEI I 2000 On the nature of aging. *Mech Age Dev117:* 93–108
24. FRANCESCHI C, VALENSIN S, BONAFE M, PAOLISSO G, YASHIN A I, MONTI D, DE BENEDICTIS G 2000 The network and the remodeling theories of aging: historical background and new perspectives. *Exp Gerontol 35(6–7):* 879–896
25. FRANCESCHI C, BONAFE M, VALENSIN S 2000 Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. *Vaccine 18:* 1717–1720
26. PAOLISSO G, BARBIERI M, BONAFE M, FRANCESCHI C 2000 Metabolic age modelling: the lesson from centenarians. *Eur J Clin Invest 30:* 888–894
27. BARBIERI M, RIZZO M R, MANZELL D, GRELLA R, RAGNO E, CARBONELLA M, ABBATECOLA A M, PAOLISSO G 2003 Glucose regulation and oxidative stress in healthy centenarians. *Exp Gerontol 38:* 137–143
28. BAUER M E 2005 Stress, glucocorticoids and ageing of the immune system. *Stress 8(1):* 69–83
29. FRANCESCHI C, CAPRI M, MONTI D, GIUNTA S, OLIVIERI F, SEVINI F, PANOURGIA M P, INVIDIA L, CELANI L, SCURTI M, CEVENINI E, CASTELLANI G C, SALVIOLI S 2007 Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mech Age Dev 128:* 92–105
30. SEN C K, PACKER L 1996 Antioxidant and redox regulation of gene transcription. *Faseb J 10:* 709–20
31. KRIETE A, SOKHANSANJ B A, COPPOK D L, WEST G B 2006 Systems approaches to the network of aging: Review. *Age Res 5:* 434–448
32. JAENISCH R, BIRD A 2003 Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genetics (suppl) 33:* 244–254
33. SCHROECKSNADEL K, FRICK B, WIRLEITNER B, WINKLER C, SCHENNACH H, FUCH D 2004 Moderate hyperhomocysteinemia and immune activation. *Curr Pharmaceut Biotechnol 5:* 107–118
34. McCLEARN G E 1997 Biomarkers of age and aging. *Exp Gerontol 32:* 87–94
35. MANTON K G 1999. Dynamic paradigms for human mortality and aging. *The Journals of Gerontol 54:* 247–254
36. ROSSOLINI G, PIANTANELLI L 2001 Mathematical modeling of the aging processes and the mechanisms of mortality: paramount role of heterogeneity. *Exp Gerontol 36:* 1277–1288
37. PIANTANELLI L, ROSSOLINI G, BASSO A, PIANTANELLI A, MALAVOLTA M, ZAIA A 2001 Use of mathematical models of survivorship in the study of biomarkers of aging: the role of heterogeneity. *Mech Age Dev 122:* 1461–1475
38. COFFEY D S 1998 Self–organization, complexity and chaos, the new biology for medicine. *Nat Med 4(8):* 882–885
39. GOLDBERGER A L 1996. Non-linear dynamics for clinicians: chaos theory, fractals and complexity at the bedside. *Lancet 347:* 1312–1314
40. KITANO H 2002 Systems biology: A brief overview. *Science 295 (5560)*: 1662–1664
41. FRIBOULET A, THOMAS D 2005 Systems biology – an interdisciplinary approach. *Biosensors and bioelectronics 20:* 2404–2407
42. LIPSITZ L A 2002 Dynamics of stability: the physiologic basis of functional health and frailty. *J Gerontol 57A(3):* 115–125
43. PERLS T, LEVENSON R, REGAN M, PUCA A 2002 What does it take to live to 100? *Mech Age Dev 123:* 231–242
44. KUSHNIRUK A W 2001 Analysis of complex decision-making processes in health care: cognitive approaches to health informatics. *J of biomed informatics 34:* 365–376