

## Updates in Pathobiology: Causality and Chance in Ageing, Age-Related Diseases and Longevity

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## State of the art

Democritus said that everything that exists in the universe is the result of chance and necessity (<http://www.normalesup.org/~adanchin/causeries/Atomists.html#N1>). Epicurus tried to visualize chance by saying that, occasionally, the normally straight paths of atoms in the universe bend a little, and the atoms “swerve.” If one considers mispairing during DNA replication, perhaps he was not far off [Luzzatto & Pandolfi, 2015].

The relationship between causality and chance is an open discussion in many disciplines. Often, the boundary among these events is thin to understand if an occurrence is related to one or to both. In particular, ageing, the related diseases, and longevity are difficult to define as consequence of causality, chance or both. Surely, it is known that longevity is based for 25% on genetic background; conditioning factors, that arise in the first part of life (socio-economic state of parents, education and month of birth, which has been found to reflect the environmental conditions during the prenatal and early postnatal period), account for another 25% of such variability; life circumstances at adult and old age (including socio-economic status and medical assistance) may account for about the remaining 50% [Caruso et al., 2012]. However, the possibility to inherit longevity increases with age: for centenaries it reaches up to 33% for women and 48% for men [Brooks-Wilson, 2013]. In any case, the concrete possibility to become centenarian, i.e. to manifest a longevity phenotype, is strictly related to the stochastic interaction, due to accidental events, with genes with a role in ageing and longevity processes [Caruso et al., 2012].

Stochastic processes are accidental phenomena due to causal factors. They play a role in physiological and pathological events, alongside genetics, epigenetics and environment. Intrinsic stochasticity in biological occurrences contributes, in fact, to the individuality of each living organism, including human beings, influencing phenotypic variability, as suggested by the role of the chance in the creation of immunological repertoire and neuronal synapses. So, probability and causality play an important role in living beings. In fact, they are subject to twofold causality: nature laws and genetic programs where Brownian random motion, disordered motion of particles present in fluids or suspensions due to the impact of water

molecules that move in different directions, and crossing over contribute to leave space for the chance. It is precisely the randomness of variability that constitutes the characteristic of evolution, hence of living beings [Kirkwood et al., 2005].

There is increasing evidence of the intrinsic stochastic nature of gene expression and macromolecular biosynthesis, since many genes are transcribed to minimal amounts of mRNA per cell, which can cause large fluctuations in biosynthesis. Genomic instability, which results in somatic mutations and chromosomal abnormalities, is another important source of intrinsic variability, as shown in aged mice, which have a mutation frequency up to  $10^{-4}$  per gene per cell. Epimutations may also occur through loss or disruption of DNA methylation patterns, affecting gene expression [Kirkwood et al., 2005; Kirkwood, 2008].

The role of stochastic processes in the ageing of individuals is clearly demonstrated by experimental studies conducted on inbred mice. They have the same genome as well as the same housing condition but show different lifespan, up to 50% higher, contrary to expectations. This proves that in living organisms there is a stochastic component that results in very little fluctuations in the genetic, epigenetic, environmental and interaction components. This involves continuous microvariations that, accumulating over time, amplify the differences between individuals, manifesting in a striking way in older ages [Kirkwood et al., 2005].

In ageing, age-related diseases and longevity, we can define chance as the occurrence of events in the absence of any obvious intention or cause. So, it must be distinguished from life circumstances that are events or facts that cause or help to cause something to happen (as an example, the death in war of a potential centenarian).

A typical example of age-related disease where chance plays a relevant role in addition to genetics and environment is cancer. The current model of cancer development is based on the notion that each of a succession of somatic mutations confers onto the mutant cell a growth advantage over normal cells in a particular microenvironment. Somatic mutations are stochastic events by their nature because they result from mispairing, which in turn originate from the equilibrium that exists in solution between tautomeric forms of the purine and pyrimidine bases. The neoplastic cell, having accumulat-

ed a number of causal mutations, is now capable of aberrant growth in a given microenvironment. There are also risk modulators such as: genetics, for presence or absence of more or less efficient alleles involved in detoxification, DNA repair, immune-inflammatory responses; diet, for presence or absence of carcinogens or antioxidants, hyper or hypocaloric diet, rich or poor in red meat; and the immune system, for efficient responses against oncogenic viruses. Thus, at the somatic cell level, oncogenesis recapitulates a Darwinian process in which mutations are the innovative force, whereas the environment selects the mutations that are advantageous. Hence, in this process, chance and necessity are strictly linked. Of course, the events might be represented by an epigenetic rather than a somatic mutation [Greaves, 2017; Luzzatto & Pandolfi, 2015].

Epigenetics is used to describe phenotypic variations that may occur in cells following a different expression of individual genes without altering the DNA sequence. These phenotypic changes are stable and inheritable from cell progeny, through DNA replication and cell division cycles. Thus, during the proliferation that occurs in the normal homeostatic replacement, a cell expresses the characteristic genes of the corresponding tissue, and not that characteristic of another one. In a broader sense, epigenetic processes are called to explain the changes in the regulation of the transcription of individual genes. Considering this broader definition of epigenetics, it is not surprising that the profound changes that occur with age in cells and tissues are also due to epigenetic processes as well as accumulation of nuclear and mitochondrial DNA mutations [Peaston & Whitelaw, 2006].

The level of complexity is very high if we consider that not only the effect of each variation but also their combinations must be evaluated. For example, lysine methylation at positions 4 and 9 in H3 has opposite effects: the first increases the expression of genes; the second reduces this action. miRNAs are also involved in gene transcription. They also regulate histone modification processes. A poor folate diet, for example, may increase the expression of specific miRNAs. Moreover, it is known that these effects might be also mediated by exogenous miRNAs, such as that from plants. So, we may hypothesize that in future we will modulate our gene expression choosing specific foods and, consequently, we will modulate ageing towards successful outcome [Bannister & Kouzarides, 2011; Dellago et al., 2017; Vaucheret & Chupeau, 2012].

How might stochastic epigenetic model underlie disease? An obvious example, as previously suggested is cancer, which arises in part from repeated changes to the microenvironment of the tissue. Most cancers arise from cycles of repeated injury and repair. Hepatocellular cancer arises directly from this process whether the injury is induced by viruses or by aflatoxin. Similarly, skin cancer arises from repeated cycles of blistering sunburn and repair. The epigenome sits at the intersection of the environment, genetic mutation and tumour cell growth. Environmental factors, such as carcinogens or diet, as well as injury and inflammation, cause epigenetic reprogramming. The epigenome also accumulates damage stochastically and through ageing [Feinberg, 2014; Sen et al., 2016].

The stochastic epigenetic model has important implications for evolutionary biology considering environmental effects that may be consistent for many generations but can then change stochastically, for example in response to an environmental crisis such as drought or famine. At this regard, during the winter of 1944–1945, a famine affected the western Netherlands. It was the result of an embargo on transport of food supplies imposed by the German occupying forces in early October 1944 in reprisal for a wave of partisan activity. It lasted for approximately 5 months and ended abruptly with the liberation by allied forces in May 1945. Several studies addressed the effects of maternal malnutrition during the different periods of gestation on health in adult life. Individuals conceived in Holland occupied by the Nazis showed at a distance of sixty years a higher incidence of diabetes, obesity and other diseases when compared to subjects age and sex matched whose mothers followed a non-famine dietary regimen in Holland occupied by allied armies. The association was present in subjects, whose exposure to famine occurred around the time of conception, proving the strict relation between causality and chance intrinsic to epigenetic changes. So, research over the past 3 decades has shown that exposure to famine during gestation has life-long effects on health, and that these effects vary depending on the timing of exposure as well as evolution of the recovery period. The effects of famine during gestation thus differ from those of adult exposure, which has only short-term effects [Heijmans et al., 2008; 2009].

## The Book

To gain insight into the role of chance and causality in ageing, age-related diseases and longevity, several papers have been assembled in these Proceedings of a Symposium held in Palermo, March 24, 2017.

Ageing affects different body tissues and is known to have a negative impact on the physiology of cells, tissues and organs, resulting in reduced functionality and regeneration capacity. It is clear that circulating factors are important contributors to the ageing phenotype. Consequently, circulating miRNAs have been carefully studied in the context of ageing in recent years. miRNAs are part of the secreted phenotype associated with senescence and are transferred by microvesicles in a paracrine manner. So, they might be an ideal target for modulating healthy ageing [Accardi et al., 2017b].

To discuss the relevance of genetics and lifestyle in the attainment of longevity, three papers mostly focused on Italian centenarians with aim to understand how to prevent and/or reduce elderly frailty and disability. In their review, Ferrario & Puca [2017] pointed out the genetic origin of exceptional longevity: healthy ageing is a complex, heritable trait that correlates with the presence of protective alleles (FOXO3A rs2802292) and lack of some of the detrimental ones (i.e. APOE $\epsilon$ 4). Furthermore, they summarized the potential problems of the genetic approaches and possible future evolution based on new technologies. The phenotypic aspects were instead approached in the papers by Bulati et al., [2017] and Accardi et al., [2017a]. In particular, in this last report the Authors discussed new approaches as bio-electrical impedance to characterize phenotypically elderly and centenarians. Centenarians were also studied in a preliminary report on triggering of Toll-like receptors in the elderly, however TLR agonists significantly enhanced the activation of dendritic cells in the peripheral blood isolated from healthy elderly, but not from centenarians, likely due to the exhaustion of immune system [Gambino et al., 2017].

Three papers by Aiello et al., [2017], Bova et al., [2017], and Vetri [2017] were devoted to the role of genetics, environment and stochasticity in chronic diseases. In particular, it was pointed out the pleiotropic nature of KIR on different diseases in that a given KIR genotype affording protection against one disease may actually predispose to another unrelated disorder [Aiello et al., 2017].

Finally, two papers concerned the pathophysiology of a typical age-related disease, i.e. cancer. In the paper by Cocciadiferro & Caruba [2017], by analysing the role of estrogens in the development of liver cancer, it was pointed out that mutations and epigenetics affect the role of estrogens. Libra & Nicoletti [2017] instead pointed out the role of life style, in particular of diet in the development of cancer.

## Conclusion

We can conclude that ageing and longevity as well as cancer as a model of age-related diseases, in themselves, are produced by a complex combination of random events and genetic background. The ageing process is driven by a lifelong accumulation of molecular damage, resulting in gradual increase in the fraction of cells carrying defects. After sufficient time has passed, the increasing levels of these defects interfere with both the performance and functional reserves of tissues and organs, resulting in a breakdown of self-organizing system and a reduced ability to adapt to the environment. It follows age-related frailty, disability and disease [Kirkwood et al., 2005; Kirkwood, 2008].

Stress, adverse environment and poor nutrition can increase the rate at which molecular damage arises. Intrinsic maintenance mechanisms, such as DNA repair and antioxidants, slow the rate of accumulation. Contributing factors are cultural, anthropological, socio-economic, sexual, linked to gender, ethnic differences, healthcare, genetics, and life occupation. In the case of nutrition, for example, a poor diet containing excess sugar and saturated fats contributes directly to the burden of damage with which cells have to deal, whereas a Mediterranean-style diet may contribute protective factors such as dietary antioxidants, a reduced amount of animal proteins and a low glycaemic index [Aiello et al., 2016; Vasto et al., 2014].

Different combinations of these events create the possibility to avoid age-related pathologies and become centenarian.





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