

GENOMIC AND DARWINIAN MEDICINE¹

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ABSTRACT Genomic and darwinian medicine deal with the application of hereditary and evolutionary principles for the understanding of health and disease. The progress in molecular and bioinformatic knowledge is making possible through a holistic approach to biological phenomena and one aspect of it, host-pathogen coevolution, is discussed with examples of research performed by our group. The search for the etiology of genetic diseases can focus on simple traits with mendelian inheritance or in more complex multifactorial characteristics, as well as in nuclear or mitochondrial DNA genes. Also important is the investigation of genetically conditioned variation in response to drugs (pharmacogenomics)

and unorthodox environmental effects (epigenetics). Every day the genome of a given cell receives one million lesions which should be repaired. Defects in repair mechanisms can lead to diseases, one important category of them being neurological disorders. The association between intronic inversions which lead to severe hemophilia A and the prevalence of Factor VIII inhibitors in these patients was also considered using information obtained by the Porto Alegre group and those of colleagues living in other cities. The final message emphasizes the need for an evolutionary approach to fully understand pathologic processes and their management. *Rev Arg Antrop Biol* 11(1):05-14, 2009.

WHAT IS GENOMIC MEDICINE?

Medicine is the art and science which aims to cure or alleviate symptoms of disease. Genomics is the scientific study of the totality of the genetic material of a species. Genomic medicine, therefore, would be the interaction between these two fields of knowledge. The progress which is occurring in the areas of molecular biology and bioinformatics can be only characterized as phenomenal, influencing all the biological sciences and with a direct reflex in medicine. The completion of the total sequence of the human genome, which occurred in 2003, can be viewed as merely a first step for the understanding of normal and pathological processes occurring in our bodies.

Different levels of study can be identified in genomic medicine (Table 1). DNA (deoxyribonucleic acid) gives the information from which a whole sequence of events will form the basic substances of a living being (proteins, lipids, carbohydrates) which will interact in a complex and integrated way through metabolic networks to (form) make the final product (phenome or phenotype) of an individual.

¹A similar text written in Portuguese was submitted elsewhere (Salzano, 2009).

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TABLE 1. Levels of study in genomic medicine

Levels	Characterization
Genome	The complete set of the genetic material (DNA) of a species.
Transcriptome	The totality of the initial product of DNA replication (mRNA).
Proteome	All the proteins of an organism.
Lipidome	The total lipid molecules of an organism.
Glycome	The set of all carbohydrates of an organism.
Metabolome	Complete set of metabolites (molecules of low molecular weight) of an organism.
Secretome	The population of gene products secreted by a cell or tissue.
Interactome	The totality of the interactions occurring in the functioning of an organism.
Regulome	The entire set of functional regulatory networks.
Phenome	The anatomical and physiological characteristics of an organism.
Epigenome	All the external factors which can influence, more or less permanently, the genetic material of an organism.

Source: King and Stansfield (2002); Pena (2004); Koonin and Wolf (2008).

WHAT IS DARWINIAN MEDICINE?

Charles Darwin (1809-1882) was the scholar who placed evolution theory in a solid scientific background especially through his masterful “The Origin of Species” (Darwin, 1859). Presently the evolutionary process is conceived as being basically determined, as Darwin postulated, by natural selection. The latter can be simply defined as the survival of the fittest and the elimination of the less adapted. Natural selection acts through two main components, viability and fertility (it is necessary to survive and reproduce in an adequate way). Of course, other factors can influence evolutionary events. Structural restrictions exist, based in the histories of individuals or species, and these histories can condition the action of random factors, as the accidental elimination of favorable variants or the fixation of neutral characteristics in small populations.

Darwinian medicine is the area of knowledge which deals with the application of evolutionary principles to the understanding of health and disease. The term can be traced back to an article published in the *Quartely Review of Biology* in 1991 by Randolph M. Nesse and George C. Williams (Nesse and Williams, 1997) and some of the questions asked are listed on Table 2. The answers to these questions undoubtedly are not easy, but they are being actively searched in researches performed all over the world; and Stearns and Koella (2008) recruited a selected group of specialists who indicated the state of the art at that time.

One of the most intriguing problems concerns mother-offspring evolutionary conflicts. A fraction of women with gestational hypertension develops preeclampsia, a condition that involves proteinuria caused by endothelial dysfunction, as well as necrosis and hemorrhage in several ma-

TABLE 2. *Questions considered by darwinian medicine*

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1. How does natural selection work? Why do our bodies show their present characteristics? What restrictions and adjustments occurred during their evolution?
 2. Why do diseases still exist?
 3. If lizards can regenerate their lost tails, why is it not possible to regenerate a lost finger in humans?
 4. If the control of cell division in multicellular organisms was developed at about 600 million years ago, why in certain circumstances this control is lost, with the onset of carcinogenic processes?
 5. Why do we have pain and fever?
 6. Why do we age?
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Source: Nesse (2004).

ternal tissues. In these cases placenta releases factors which may cause maternal endothelium dysfunction. If the problem develops before the completion of the gestational period a dilemma arises: the longer gestation is maintained, the less are the infant risks, but the greater the maternal morbidity and mortality risks. There are two explanations for the pathology; the first is that it arises as a side effect of abnormal physiological processes that would be maladaptive for mother and fetus as well. But the alternative is that preeclampsia is maladaptive to the mother but adaptive to the fetus. The function of the placental factors responsible for the maternal endothelial dysfunction would be to increase the blood flow to the placenta, what would be important for fetuses with growth restriction. The evolutionary perspective does not immediately suggest better treatment for the disease, but can encourage new hypotheses about its proximate causes (Haig, 2008).

SYSTEMS BIOLOGY

Systems biology attempts to achieve an integrated understanding of life forms at multiple levels. Characteristically, it involves large-scale measurement, such as a whole cell or even an entire multicellular organism. The development of molecular and bioinformatic tools led to the possibility of investigating the whole set of entities of a certain class found in an organism, tissue, organ or cell. Complex phenomena as those considered by these approaches can be analysed in the form of a network, composed by nodes and the connections between them, the edges. Evidence has been obtained that a gene's centrality in a network could constrain its rate of evolution. But the amount of variability of a given genetic region seems to depend on several other variables, such as the levels of expression of its units, and the degree of their dispensability. The most important

genes would be those that are highly expressed, interact with many other proteins, have multiple paralogs, show strong fitness reduction upon knockout, are rarely lost during evolution, and evolve slowly at the sequence level (Koonin and Wolf, 2008).

This scenario emphasizes the *status quo*. But Camps et al. (2007) contended that a convergence of directed evolution, population genetics, genomics analysis, and experimental adaptation studies (experiments) indicated that positive selection (the favoring of the new-variants that increase the fitness of an organism) is a fundamental driver of evolution. The positively selected mutations are of two kinds, those that directly affect enzymatic functions, and compensatory variants, which suppress pleiotropic, slightly deleterious effects of the former.

Duarte et al. (2007) have made a global reconstruction of the human metabolic network based on genomic and bibliomic information. They identified 1,865 genes which were associated with 3,623 enzymes and 3,673 reactions, making possible: (a) to identify where important gaps of information occur for the global knowledge of these processes; (b) the formulation of *in silico* models; (c) evaluation of the implications of intracellular compartmentalization; and (d) clues for the potential use of correlated reaction sets for drug target identification.

In a similar approach, Goh et al. (2007) devised what they called the human disease network, examining the relationships among the genetic disorders. They verified that of 1,284 conditions 867 had at least one link to other diseases, and 516 of them formed a giant component. On the other

hand, 1,377 of 1,777 disease genes were connected with others of the same type, and 903 clustered in a single block. The number of genes associated with a given disorder could be high: 41 for deafness, 37 for leukemia, and 34 for colon cancer.

HOST-PATHOGEN COEVOLUTION

Some of the most elegant examples of ongoing evolution are provided by host-parasite coevolutionary relationships. In its most strict sense we can only characterize a situation as coevolutionary when one of the species in interaction varies in genetic composition in response to a genetic variation in the other(s). Many factors are involved in the host-pathogen relationships. For both organisms size, rate of reproduction, life histories, and genetic variability matters. Interactions between the host immune system and the pathogen virulence are of utmost importance, as well as the type of the target organs and parasite density in them, the occurrence of single or multiple agents of disease, and in the latter case the competition between them. Biotic or abiotic vectors provide additional variables that should be taken into consideration. This multitude of sources of variation, of course, make quantitative analytic treatments difficult. But for recent analyses about the evolution and expression of virulence see Ebert and Bull (2008) and Koella and Turner (2008).

Domínguez-Bello et al. (2008) have investigated the multilocus sequences from seven housekeeping genes of *Helicobacter pylori*, a human gastric indigenous bacteria which may cause gastric cancer and peptic ulcer disease. The study involved material

from Africans, Europeans, Asians, Amerindians, and South American Mestizos. Strains that had been cultured from Africans, Europeans, and Asians were all characteristic of people from these continents only. However, Amerindians and Mestizos carried mixed strains: *hspAmerind* and *hpEurope* were found in Amerindians, and *hpEurope* and *hpAfrica1* in Mestizos. A comparison was made between the genetic diversity of these strains with those of the mtDNA of 1,148 people from the same or nearby populations. The least genetically diverse *H. pylori* strains were *hspAmerind*, isolated from the most homogeneous human populations. On the other hand, *hpEurope*, highly diverse, seems to be expanding its host range. Coevolutionary relationships are clear.

Another pathogen, human herpesvirus type 8 (HHV-8), also called Kaposi sarcoma-associated herpesvirus, is the etiological agent of all forms of this sarcoma, primary effusion lymphoma, and certain lymphoproliferative disorders. Not all affected, however, develop these conditions, indicating virus-host synergism. Antibody prevalences against HHV-8 are low in western populations (less than 5%), they are higher in Africa, but the highest reported were found in Amerindians. Souza et al. (2009) recently obtained data from 760 individuals from 11 Brazilian, Bolivian and Paraguayan Amerindian populations, comparing them with those of 2,479 subjects from 26 indigenous groups of Brazil, French Guiana, and Ecuador. The range of frequencies was surprisingly high (0%-83%); factors which may be influencing this variability are: (a) Host and virus genotypes; so far only HHV-8 sub-

type E was found in Amerindians, but the number of studies considering this point is small; or (b) Environmental variables such as time which elapsed from the virus first introduction into the population, preferred routes of transmission, human population density and mobility, as well as behavioral characteristics favoring or preventing transmission.

Mycobacterium tuberculosis has been a human pathogen for millennia. Molecular studies indicated that the *M. tuberculosis* complex (including also *M. canettii*, *M. microti*, *M. bovis*, and *M. africanus*) should have had an African origin may be 2.5 million years ago. The American pathogen was identified as member of this complex, and should have been present in the prehistoric peopling of the continent. But osseous evidence for tuberculosis was only observed in remains of approximately 300 AD (Wilbur et al., 2008).

Infection with *M. tuberculosis*, an intracellular pathogen, results in disease in only 5%-10% of those exposed; the others remain unaffected. Most of them develop a delayed-type hypersensitivity response two to four weeks after infection, manifested by a positive response (skin induration) to intradermal injection with purified protein derivative (PPD) from the bacteria, the Tuberculin Skin Test (TST).

The Aché of eastern Paraguay had never seen tuberculosis before recent contact with other groups and had no word for the disease in their language. A longitudinal study since the inception of their exposure to the pathogen verified that in fewer than 15 years 18% of the population had been diagnosed with active tuberculosis (Hurtado et al., 2004).

The lack of skin induration after the PPD intradermal injection is defined as anergy and may occur due to variability in a group of small proteins, cytokines, involved primarily in communication between cells of the immune system. The Xavante Amerindians of central Brazil are being studied by our group for half a century now (review in Coimbra et al., 2002) and show a high rate of anergy even after newborn BCG vaccination. To investigate this phenomenon, Zembrzuski et al. (2009) have performed a detailed investigation of 19 polymorphisms in 15 genes related to the immune response from almost all individuals (481) of the *Etéñitépa* village. Anergy was observed in 69% of the subjects, and polymorphisms in four genes (*SP110*, *PTPN22*, *IL12RB1*, and *IL6*) were absent or showed very little variability, in contrast with findings in other ethnic groups. Three others (*IFNG*, *IL4*, and *IL10*) were significantly associated with response to TST. In tribal groups such as the Xavante exposure to a variety of infections and trauma, associated with specific genetic factors, may disturb the T-helper 1 and T-helper 2 balance, leading to increased immunological susceptibility (Hurtado et al., 2004). The public health importance of studies like the one reviewed here for the health of communities living at this level of socioeconomic development is obvious.

SEARCHING FOR THE ETIOLOGY OF GENETIC DISEASES

A large number of common diseases has a multifactorial etiology, associated to genetic and environmental characteristics. Since natural selection has acted for

thousands of years in our genome, genetic risk factors should be related to low frequency alleles. Genome analyses for the identification of these factors involve non-trivial costs, but technological progress is making possible the testing of nothing less than 500 thousand SNPs (single nucleotide polymorphisms) in a single experiment! As a consequence, important progress occurred in the understanding of the etiology of complex diseases, like type 2 diabetes mellitus (of late onset), myocardial infarction, prostate cancer, and obesity (Ropers, 2007).

On the other hand, only about two thousand of the 25 thousand protein-coding human genes and practically none of the non-coding genes were related to diseases, and causal mutations are known for only 3,345 gene-mapped genetic illnesses. Since familial recurrence not always occurs in genetic diseases (example: autosomal dominant severe diseases of early onset), only a fraction of them is known. In the mouse, where knockout experiments can be done, only 3% to 4% of the mutations do not show a phenotypic manifestation (Ropers, 2007).

A special class of diseases are those due to genetic changes in mitochondrial DNA (mtDNA). The organism which gave origin to our mitochondria invaded an eukaryotic cell two to three billion years ago. In the course of evolution many of their genes (about 1,500) were transferred to nuclear DNA, only 37 remaining in the organelle. These genes are basically related to oxidative phosphorylation, transforming diet calories in usable energy and secondarily generating toxic oxygen reactive substances. Our hunter-gatherer ancestrals needed high

doses of energy; mutations that would increase such doses would be favored at that time. Presently, however, with the control of environmental stimuli and high-caloric diets, such mutations turned to be harmful. The association between mtDNA mutations and diseases involve clinical manifestations which affect the brain, heart, skeletal muscle, kidney, and endocrine system, the same tissues that are affected by aging. Specific symptoms related to these mutations are blindness, deafness, locomotion problems, dementia, cardiovascular disease, muscular weakness, renal dysfunction, and diabetes (Wallace, 2005).

PHARMACOGENOMICS

This area of knowledge deals with genetically conditioned variation in response to drugs. In the beginning the emphasis was placed in monogenic characteristics (pharmacogenetics). With the possibility of studying many genes simultaneously we are presently in the pharmacogenomics era (Hutz and Fiegenbaum, 2004).

The action of drugs can be influenced in different ways: (a) in its metabolization; (b) in membrane transport; (c) in target genes for drug action (for instance, receptors). Drug metabolism generally occurs in two steps: phase 1 involves the introduction of small polar groups which increase aqueous solution solubility. At this stage the main genes involved are those which act in the P450 monooxygenase cytochrome superfamily (CYP450). Phase 2, on the other hand, includes the conjugation with acetate, inorganic sulphate, sugars or amino acids. The respective genes act on different series of enzymatic superfamilies

(example: genes which act on glutathion S-transferase).

MDR-1 (multidrug resistance gene) is one of the most studied among the ABC family of transporters (*ATP-binding cassette*) and codifies a membrane transmitter, P glycoprotein, which is very important for substrate availability; and at least 15 *MDR-1* polymorphic SNPs were detected. As for genes that could be targets for drugs, *ECA's* variability could be important in the response to inhibitors of the angiotensin conversion enzyme (Hutz and Fiegenbaum, 2004). The final objective of these studies is individualized therapy; the drug's dose would be adjusted to each person in particular.

EPIGENETICS

The term refers to the study of the mechanisms by which genes lead to phenotypic effects. Cell clones could inherit phenotypic changes which are not due to nucleotide variations. DNA-linking proteins codified by regulatory genes sometimes produce epigenetic changes which could be preserved during the mitotic divisions in somatic tissues. Generally, however, meiosis erases these marks, and therefore the species ontogenetic program starts again in each generation. In multicellular organisms, therefore, these epigenetic processes are especially important with relation to somatic mutations.

Gene expression is intimately connected to the addition of methyl groups to specific sites of the DNA molecule. Their absence is associated with transcription, their presence with gene silencing. Different degrees of DNA methylation in sperma-

togenesis or oogenesis lead to differential expression of symptoms in certain diseases, whether the transmission occurs via paternal or maternal inheritance (the phenomenon called genomic imprinting). One of the classical examples is the 15q11-q13 deletion, which when transmitted by the father leads to Prader-Willi syndrome, and when transmitted by the mother to the quite different Angelman syndrome. This effect is due to a center of genomic imprinting located in this region. It should be noted, however, that there are other causes for these two syndromes. Another heterogeneous condition, autism (characterized by the patient's detachment from the external world and interpersonal relationships) can also have as one of its causes epigenetic phenomena (Beaudet, 2008).

DNA REPAIR AND NEUROLOGIC DYSFUNCTION

DNA damage is one determinant of cell dysfunction and death, carcinogenesis, and the aging process. Every day the genome of a given cell receives one million lesions! If these lesions are not repaired, mutations of several types, gene expression disturbances, or the formation of detrimental proteins may occur.

To cope with these problems cells are equipped with a large number of repair enzymes and pathways. Defects in them may lead to apoptosis (death) or cell transformation; and recently special attention was given as how these processes are reflected in neurological diseases.

DNA damage sources can be endogenous, caused by products or subproducts of the cell metabolism (for instance reac-

tive oxygen species, chelating agents and aldehydes); or exogenous, determined by physical (example: ionizing radiation) or chemical (heavy metals derived from pollution, or aflatoxin, found in the food) agents which occur in the environment.

Examples of neurodegenerative diseases due to DNA repair defects are certain types of ataxias (loss of body movement coordination) and xeroderma pigmentosum (a dermatologic affection). The pronounced sensibility of non-dividing neuronal cells to DNA damage can be explained by: (a) their reliance on replication-independent repair pathways for lesion removal; and (b) their high metabolic requirements with the associated oxidative burden (Kulkarni and Wilson, 2008).

SEVERE HEMOPHILIA A AND INTRONIC INVERSIONS

Hemophilia A is a hemorrhagic disease caused by a wide variety of changes in the Factor VIII coagulation gene. This gene is located in chromosome X's long arm (Xq28) and is extremely complex. Its size totals 186 thousand base pairs, with 26 exons (codifying units). The latter, however, constitute just 5% of the gene, the remaining being formed by introns (regions situated between the exons).

Frequent causes of severe hemophilia A are two inversions which occur in introns 22 and 1 of the gene. Studies performed by our group (Leiria et al., 2009) verified, in 107 patients of this type, prevalences respectively of 46% for inv. 22 and 3% for inv. 1. Comparisons with series involving 3,871 severe hemophiliacs in 15 countries showed an extreme homogeneity (for inv.

22, 40-49% in 14 of the 21 series with at least 31 patients tested, distributed by 13 nations).

In contrast with these findings, the prevalence of Factor VIII inhibitors, one of the main therapeutic problems for these individuals, showed ample heterogeneity among inv. 22 carriers (5-51% in seven countries, 1,482 patients). The highest value was found in our series. This variability is due to many factors. Besides inv. 22, the type of Factor VIII that these patients receive, von Willebrand factor, which acts as an immuno-coadjuvant, as well as other characteristics of their immune system, should also be important. Our group is starting studies in these latter characteristics, to better analyse the causes of this process.

FINAL MESSAGE

We are currently in the post-genomic era, in which DNA structural variation is related to a wide spectrum of phenomena that are manifested at the functional level. The search is for a synthetic vision, that could relate these different phenomena among themselves. Pathological processes can only be understood through an approach which transcends immediate facts, looking for an interpretation in the wide context of organic evolution. It is through this investigation that we eventually could establish in an appropriate way our position in the universe.

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