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COMMENTARY

Old-style epidemiology and epi*genetic*demiology

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Epidemiology has widely changed in the past decades. When we started our first population-based epidemiological studies,[1] collecting historical, physical and biohumoral data from a considerable number of representative unselected subjects was good enough. Our CArdiovascular STudy in the ELderly (CASTEL),[1] our Mirano[2] and Camposampiero[3] projects produced several papers and gave important results in terms of prevention of arterial hypertension and arteriosclerosis and in terms of prevalence of many diseases in our country. Their prospective parts also provided interesting information about incidence and prevention of these diseases.[4]

Years later, when we launched the LEOGRA study (Last Evidences Of Genetic Risk factors in the Aged), cardiovascular epidemiology had already evolved into a new discipline, requiring the use of instrumental devices aimed at measuring pulse wave form and velocity, cardiac index, capillaroscopy, the cognitive pattern and so on. Old-style epidemiology is now confined to very simple studies or to those performed in the 3rd World.[5] But above all, 'genetics' made its triumphal entrance into epidemiology. And our ongoing GOLDEN study (Growing Old with Less Disease Enhancing Neurofunctions) is entirely based on genetics.

The reasons for this escalation and for the aggressive entrance of genetics in the world of cardiovascular epidemiology are the chronic diseases—such as arterial hypertension, arteriosclerosis, impaired aortic fitness and so on—that turned out to be elusive showing an ambiguous nature and the classic old-style epidemiology proved to be insufficient to investigate the great epidemics of 20th and 21st centuries, that is, the degenerative ones.

To tell the truth, epidemiologists were not conceptually ready to tackle this challenge, and they did not have at their disposal the necessary cultural tools. As a matter of fact, except for sporadic cases, epidemiologists and geneticists worked separately, and the former continued thus far to apply to population genetics the tools that were typical of old-way epidemiology. As a consequence, they often obtained contrasting results. In the last majority of cases, cardiovascular epidemiology simply incorporates one or more new variables represented by polymorphisms of some genes, then searching for associations between these polymorphisms and old-style variables. This procedure was sometimes leading to something coherent, while in other cases, comprehensibly, it failed.

An important cause of failure is represented by the fact that—apart from a limited number of heritable rare diseases—human genetics is not Mendelian in its nature, as chronic degenerative diseases are not caused by a single gene or influenced by a single polymorphism, but are rather the consequence of the effects of a mosaic of dozens of genes. Not only this, but the genetic expression in a single, specified, particular man is also strongly influenced by environmental conditions (sodium intake, ethanol and coffee drinking, dietary habits, smoking, environmental temperature, seasons, marital status, lifestyle and so on[6]). Then what we observe in each specific case is the resultant of different interactions. When we deal with cardiovascular epidemiology, the genetic pattern might be simply considered nothing more than one additive risk factor.

The interaction between genes and environment, often defined as 'the echogenetic context',[7] makes the analysis of risk more complex, as many covariables (both genetic and environmental) have to be taken into consideration in the multivariate equations of risk.

Lifestyle is a typical example of echogenetic interaction. This has been clearly shown in the frame of the EPOGH (European Project on Genes in Hypertension), where sodium intake,[7,8] ethanol drinking,[9] geographic location and other conditions or activities have been shown to interact strongly and significantly with gene expression. For instance, in about a thousand unselected men and women from three European countries where the frequency of the β-adducin T allele and salt intake differed across populations, both variation in genetic background and sodium consumption explained the observed heterogeneity in the phenotype–genotype relationships.[7] In a similar setting, alcohol intake modulated the association between the PPAR2 *Pro12Ala* and high-density lipoprotein cholesterol level.[9] As well, in the LEOGRA study menopause and the *C825T* polymorphism of GNB3 gene contributed to the fat distribution in women,[10] and in the GOLDEN study years of education strongly modified the effect of the above mentioned polymorphism on cognitive pattern.[11]

A further confounding factor for epidemiologists dealing with the putative genetic determinants of arterial hypertension is *epigenetic*. As old-style researchers, we have been used to think that everything comes from the genome and nothing goes to the genome, but, if epigenetic is a reality (and it is), we have to consider that the environment in general not only influences the effect of a specific gene like an unsuspected travel companion, but also modifies the gene expression through an action on a promoter gene. In

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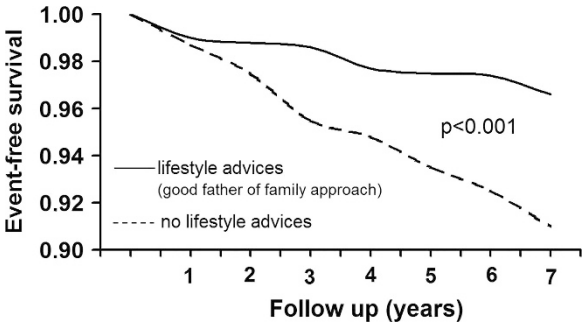

Figure 1 The 'good father of family approach' (—), an environmental intervention reducing the risk of stroke among 1189 unselected men and women from the cardiovascular study in the elderly. Active advices concerning lifestyle lead to better stroke-free survival in comparison to subjects not receiving advices (---).[4]

practical terms, this action is represented by DNA methylation.

Today, there are clear evidences that hypertension regulation is influenced by epigenetic variation, that the epigenetic process represented by the DNA methylation is of paramount importance in regulation of expression of some genes-regulating blood pressure, and that the DNA methylation is largely environment dependent. This could just be the mechanism (or one of the mechanisms) explaining the gene-environment echogenetic context.[7–9]

In the paper the interactions between drinking and DNA methylation of ADD1 gene promoter modify the essential hypertension susceptibility in a population-based case-control study by Han *et al.*[12] is in line with this view. In about a thousand of population-based cases and controls, the authors were initially unable to find any associations between the single nucleotide polymorphisms *rs3755885*, *rs2071694*, *rs4963* and *rs3775067* of the gene codifying for α-adducin and arterial hypertension. The search for such genotype/phenotype associations is often disappointing, but in this research the authors used the free generalized multifactor dimensionality reduction method to explore the role of DNA methylation on the potential effect of the above-mentioned polymorphisms and susceptibility to arterial hypertension, after environmental factors, including alcohol drinking, were taken into account in the model. In this way they found that the model T-carriers vs GG of rs4961 exerted a significant protective action against the development of arterial hypertension after adjusting for the predictive factors represented by serum triglycerides, body mass and alcohol drinking.

The plausibility of ethanol as a possible DNA methylator has been previously demonstrated by other authors in experimental rather than in human setting. Lower DNA methylation could be associated to higher adducin gene promoter expression, and the Na^+/K^+-ATPase pump could be the intermediate phenotype. The reasons why alcohol consumption leads to blood pressure rise are many, and this could be one of them.

To be more precise, ethanol consumption does not have the same effect in everybody. It is well known that some people respond to ethanol with hypertension or hypercholeterolemia[9] while other seems to be free from these complications. This different behavior probably rests on a different genetic pattern. For instance, we found in the EPOGH context that subjects who are Ala-carriers for the PPAR2 *Pro12Ala* gene respond to alcohol drinking with a rise in low-density lipoprotein cholesterol, while those with the Pro/Pro genotype do not.[8] The response to drinking is therefore depending on a lot of genetic factors interacting with each other and with environment. This is presumably true for other lifestyle characteristics too. As a consequence, the so called 'good father of family approach'[4] (Figure 1) should be based on the net resultant of effects on subjects genetically predisposed to respond or not to respond to different, particular, selective actions (to stop smoking or drinking, to reduce dietary fat, to lose body weight, to increase fiber intake and so on).

Essential arterial hypertension (and probably many other chronic diseases) are therefore multifactorial in nature, and are influenced by genes, by environmental factors and above all by their interactions mainly mediated by DNA methylation. To search for heritability, it is therefore necessary to explore the gene-environment interactions through the epigenetic epidemiology.

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